last 20 minutes (*15, 16*). Clearly this animal cannot be utilizing its stored oxygen as a long-term reserve, but may be drawing on it to withstand the short periods of anoxia encountered in the rapidly fluctuating vent environment. The high metabolic rate of *R. pachyptila* and the high concentrations of hemoglobin in its blood may reflect a high demand for oxygen for H_2S metabolism.

The high oxygen carrying capacity, high hemoglobin cooperativity, and high oxygen affinity of the whole blood of *R. pachyptila* should enable the animal to load oxygen into the blood and to transport it to the trophosome tissue at a substantial rate. This high concentration of oxygen in the blood may also serve as a short-term oxygen store for use during adverse conditions. The moderate thermal stability of the hemoglobin oxygen affinity is useful as the pigment is thermally tolerant enough so that temperature changes will not radically alter its properties, and yet it is sensitive enough so that the higher temperatures, which are found at the base of the animal, may allow for enhancement of oxygen unloading at the trophosome. The small interaction between CO_2 concentration and oxygen affinity is of adaptive value for an animal which must transport CO_2 to the tissues along with oxygen. In summary, the blood of *R. pachyptila* has a combination of characteristics that would be supportive of a chemoautotrophic animal in an unusually variable environment.

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7. The Hem-O-Scan gives accurate readings for human blood over a range of temperatures and *p*H values. Our instrument is modified to prevent excessive oxygen leakage in the sample compartment, insulated to prevent condensation at lower temperatures, and equipped with an air pump and control valve for slow oxygen introduction. Dehydration of the sample is prevented by the use of a double Teflon membrane instead of the single copolymer membrane recommended by Aminco for sample preparation. The Hem-O-Scan is equipped with interference filters that transmit at 560 and 576 nm [D. A. Powers, H. J. Fyhn, U. E. H. Fyhn, J. P. Martin, R. L. Garlick, S. C. Wood, *Comp. Biochem. Physiol.* **62A**, 67 (1979); G. N. Lapennas, J. M. Colacino, J. Bonaventura, *Methods Enzymol.*, in press.
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15. Two small vestimentiferan worms (about 20 g of wet tissue) were recovered alive (December 1979, Rose Garden animals) and placed in a running water, pressure aquarium system at 130 atm and 12°C [L. B. Quetin and J. J. Childress, *Deep-Sea Res.* **27A**, 383 (1980)]. They soon extended their plumes and apparently remained healthy until killed 2 weeks later. Two days after capture one of these worms was placed in a pressure vessel respirometer [12°C, 250 atm; R. Meek and J. J. Childress, *ibid.* **20**, 1111 (1973)]. This animal consumed oxygen at a rate of 0.13 μl of O_2 per milligram (wet weight) per hour. This consumption rate is similar to that of the pogonophoran *Siboglinum ekmani* of 0.06 and 0.12 μl of O_2 per milligram (wet weight) per hour, previously measured by C. Little and B. Gupta [*J. Exp. Zool.* **51**, 759 (1969)] and by C. Manwell, E. Southward, and A. Southward [*J. Mar. Biol. Assoc. U.K.* **46**, 115 (1966)], respectively. However, since these pogonophorans are minute compared to *R. pachyptila*, the rate for the vestimentiferan is effectively much higher.
16. With a respiratory rate of 0.13 ml of O_2 per 100 g (wet weight) per hour, a hypothetical, 100-g animal would have an oxygen consumption rate of 13 ml of O_2 per hour. If 80 percent of the animal is coelomic fluid with an oxygen carrying capacity of 5.4 ml of O_2 per 100 ml of blood, the total amount of oxygen the animal would be able to store would be 4.5 ml (80 percent × 5.4 ml of O_2 per 100 ml of blood). With a respiratory rate of 13 ml of O_2 per hour this is only enough O_2 to last about 20 minutes (4.5 ml of O_2 per 10 ml of O_2 per hour = 0.35 hour, ~ 20 minutes).
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Hemoglobin Kinetics of the Galápagos Rift Vent Tube Worm *Riftia pachyptila* Jones (Pogonophora; Vestimentifera)

Abstract. *Kinetics of the reactions of* Riftia pachyptila *hemoglobin with oxygen were followed spectrophotometrically by stopped-flow and laser flash photolysis techniques. The rate of oxygen dissociation increases eightfold over the range of 5° to 20°C (*k = 2.2 sec^{-1} *at 10°C). Oxygen recombination after flash photolysis was biphasic. The rates of both slow and fast phases of the reaction were independent of temperature from 0° to 20°C (*$k'_{fast} = 7 \times 10^6$; $k'_{slow} = 1 \times 16^6$ liter $mole^{-1}$ sec^{-1}*). As the oxygen affinity is relatively temperature independent, analysis in terms of the two-state model of cooperativity requires that the conformational equilibrium constant* L *decrease by about 50-fold between 3° and 15°C.*

Submarine thermal springs and hydrothermal vents along the central valley of the Galápagos Rift, at a mean ocean depth of about 2500 m (*1*), are the sites of numerous animals. Conspicuous among these is the tube worm, *Riftia pachyptila* Jones (*2*), belonging to the order Vestimentifera of the phylum Pogonophora, which exceeds 1.5 m in length and lacks both mouth and gut (*2*). The blood in these worms functions primarily as a carrier of oxygen and hydrogen sulfide to the trophosome where symbiotic bacteria generate energy by the oxidation of hydrogen sulfide (*3–6*).

Electron microscopy reveals that the hemoglobin molecules are two-tiered hexagonal arrays of submultiples of molecular weights 15,000 and 30,000, typical of annelid hemoglobins (*7, 8*). The expected molecular weight is about 3×10^6 but *Riftia* hemoglobin seems to dissociate in dilute solution, the apparent molecular weights being 1.7×10^6 and 0.4×10^6 (*7*).

The oxygen affinity is moderately high, is independent of *p*H and carbon dioxide concentration, and is weakly dependent on temperature (*6*). Oxygen binding is cooperative with a Hill constant of $n = 2.5$ to 3.0 (*6*). We examine here some of the reactions of *Riftia* hemoglobin with oxygen.

A specimen of *Riftia pachyptila* (USNM 59968) was collected by the submersible *Alvin* on 15 December 1979. Blood was drawn from the dorsal vessel and was found to contain 5200 μmole of heme per liter (*9*). The heme content of the blood is equivalent to an oxygen binding capacity of 11.6 percent (by volume), about half that of human blood (*6*).

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Oxygen dissociation from blood was measured by rapid mixing of oxyhemoglobin with solutions of dithionite in a Gibson-Durrum stopped-flow apparatus (*10*). The rate of dissociation increases 10- to 20-fold from 5° to 25°C (Fig. 1). The measured first-order rate constants were 1.1, 2.2, 4.4, and 9.0 per second at 4°, 10°, 15°, and 20°C, respectively.

The rate of recombination of oxygen after laser flash photolysis of oxyhemoglobin was measured spectrophotometrically at 436 nm (*11*). The reaction is biphasic with second-order rate constants of 1×10^6 and 7×10^6 liter mole^{-1} sec^{-1} (Table 1). In contrast to the oxygen dissociation velocities, these rates were independent of temperature between 0° and 25°C.

There are indications of a chemical rather than an allosteric origin for the kinetic heterogeneity. In Fig. 2, the proportion of fast and slow recombination reactions is independent of the extent of photolysis. In an allosteric model the proportion of rapid reaction would be expected to increase at low fractional photolysis.

There are, however, analogies available (*12*) that suggest multiple long-lived conformations, and we have observed that omitting NaCl from the medium affects both the rates and proportions of the two species. Dissociation into subunits is also a possibility.

Allosteric analysis of results. The results may be explained in terms of the two-state model of cooperativity (*13*). In this model, a hemoglobin molecule may exist in either a low affinity (T) state or a high affinity (R) quaternary conformation, related by the allosteric equilibrium constant L. Deoxyhemoglobin is predominantly T state, whereas oxyhemoglobin is primarily R state. As deoxyhemoglobin becomes increasingly saturated with ligand, the hemoglobin will at some partially liganded stage, depending on conditions, switch from the T to R quaternary conformation. In order to apply the model, the binding and dissociation rate constants for both the T and R states are required. Our analysis requires that the R to T switch after photolysis is faster than recombination with oxygen, and experiments with carbon monoxide support this (*14*). It is important to note that binding rate constants are second order and therefore concentration dependent, whereas dissociation rate constants are first order and concentration independent.

After full photolysis of oxyhemoglobin, at high oxygen concentration (250 μM), the hemoglobin molecule relaxes from R to T, within 100 μsec, then proceeds to react with oxygen. Initial binding of oxygen is to the T state, and since the dissociation rate does not contribute greatly to the observed relaxation at high oxygen concentration, estimates of the T state rates involved are available. Oxygen rebinding to the R state may be observed by partial photolysis (~10 percent) of oxyhemoglobin. The rate of recombination of oxygen shows a difference of only 1.5-fold between full breakdown (T state) and partial breakdown (R state). At low initial saturations (~10 percent), hemoglobin (45 μM) is in excess. In this instance, the dissociation rate plays a major role in the relaxation that follows full photolysis. An estimate of the T state dissociation rate constant is available by comparison of the actual T state rates observed at high oxygen with those at low oxygen. With oxygen binding rates of 7×10^6 liter mole^{-1} sec^{-1} and 1×10^6 liter mole^{-1} sec^{-1}, the dissociation velocities from the T state become 2×10^3 sec^{-1} and 2.5×10^2 sec^{-1}, respectively. Remarkably, there is no evidence of a temperature effect on oxygen dissociation from the T state.

When R state combination rates are taken together with the dissociation velocities measured with the use of dithionite (attributed to the R state), estimates of both R and T state affinities are available, and the form of the oxygen equilibrium curves may be calculated. The experimental P_{50} values at 3° and 15°C (*6*) are reproduced with $L = 1 \times 10^4$ and 2×10^2, respectively. Hill's n is 2.1 at 15°C,

Table 1. Relaxation rates for the combination of oxygen with *Riftia* hemoglobin as a function of temperature. The progress of the reaction can be resolved into slow and fast phases.

Temperature (°C)	Relaxation rates (10^{-2} sec^{-1})					
	Fully oxygenated				Partially oxygenated; full photolysis	
	Full photolysis		Partial photolysis			
	Slow	Fast	Slow	Fast	Slow	Fast
0	2.0	17	1.9	28*	2.7	32
5	2.7	17	2.2	17		
6.5					2.4	32
10	2.8	19	1.8	35*		
12					3.9	37
15	3.3	23	3.3	23	3.9	39
20	2.9	17	4.2	28		

*Approximate values.

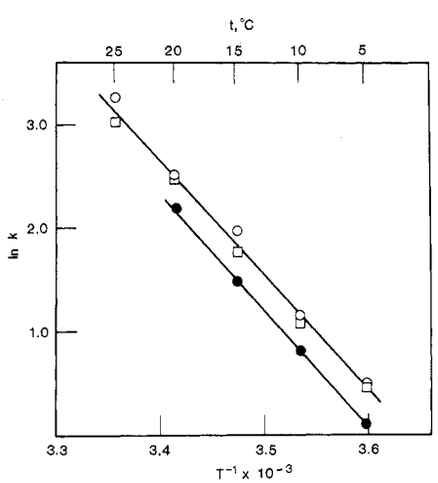

Fig. 1. Rate of dissociation of oxygen from *Riftia* hemoglobin as a function of temperature. (●) Blood hemoglobin in 0.05M potassium phosphate buffer, pH 7.5, containing 0.2M NaCl; (○) blood hemoglobin in 0.05M potassium phosphate buffer, pH 7.5; (□) coelomic fluid hemoglobin in 0.05M potassium phosphate buffer, pH 7.5. All solutions contained 1 mM EDTA.

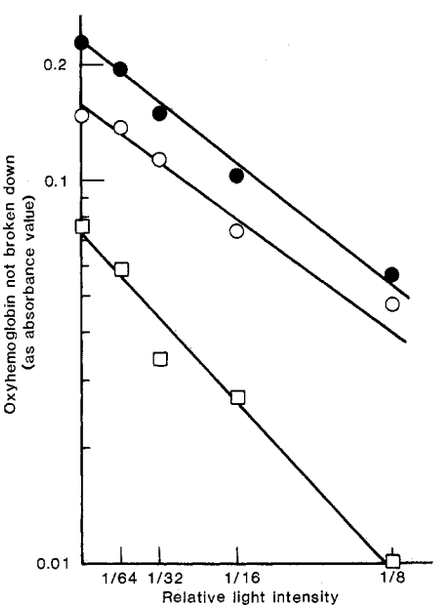

Fig. 2. Apparent quantum yield of photolysis of *Riftia* oxyhemoglobin. The figure shows the amount of oxyhemoglobin remaining undissociated as a function of light intensity; 45 μM hemoglobin (as heme), 436 nm, 2-mm cell, 20°C. The apparent quantum yield is 0.06 in relation to carbon monoxide myoglobin taken as 1.0. Amplitudes derived from a least-squares fit of the recombination reaction to a sum of two exponentials. (●), Total reaction; (○), slow component; (□), fast component.

in fair agreement with the value of 2.5 found in equilibrium measurements at 25°C.

The amount of data so far available is too small to permit great confidence in the numbers used for the allosteric analysis, but they do show that an internally consistent description is possible.

The velocity constant for dissociation of oxygen from *Riftia* hemoglobin is within the range commonly encountered for many hemoglobins (*15–17*), as is the absolute value of the velocity constant for combination of *Riftia* hemoglobin with oxygen (*15*).

Riftia normally encounters temperature fluctuating from 2° to 23°C at the mouth of the vent. Arp and Childress (*6*) report that the oxygen affinity of *Riftia* hemoglobin changes less than twofold over the range 3° to 14°C. The combination rates change not at all over this range. The relative insensitivity of the oxygen equilibrium to temperature in the face of a drastic increase in the dissociation rate can be compensated for only by a large decrease (50-fold) in the value of L, the conformational equilibrium constant. This effect of temperature on L is in the same direction as has been reported for trout I hemoglobin (*18*) and for menhaden hemoglobin (*19*) and again suggests that *Riftia* hemoglobin is similar to the few others that have been examined in this respect.

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Carcinogenicity in Mice of Mutagenic Compounds from a Tryptophan Pyrolyzate

Abstract. *The compounds 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole and 3-amino-1-methyl-5H-pyrido[4,3-b]indole, which are potent mutagens in a tryptophan pyrolyzate, are hepatic carcinogens when given orally to mice at concentrations of 200 parts per million in a pellet diet. Female mice showed higher susceptibilities to both compounds than male mice.*

On the basis of the finding that mutagens are formed in the charred parts of broiled meat and fish (*1–4*), series of new heterocyclic amines in pyrolyzate of amino acids, proteins, and proteinaceous foods were found to be highly mutagenic to *Salmonella typhimurium* TA 98 and TA 100 (*5–10*). In fact, some of these compounds have higher specific mutagenic activity than aflatoxin B_1. Among these new heterocyclic amines, which are being subjected to in vitro and in vivo carcinogenicity tests, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]-indole (Trp-P-2) from tryptophan pyrolyzates and 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) from glutamic acid pyrolyzate have been shown to be carcinogenic in an in vitro transformation system with embryonal Chinese hamster cells (*11–13*). Moreover, Trp-P-1 induced fibrosarcomas locally when injected subcutaneously into hamsters and rats (*14*). We report that Trp-P-1 and Trp-P-2 are carcinogenic to mice and

Table 1. Incidence of hepatic tumors in mice fed on diets with Trp-P-1 or Trp-P-2 (200 ppm) for up to 621 days.

Treatment	Sex	N*	Number of mice with hepatic tumors				
			Hepatocellular tumor		Hemangioma	Total	P‡
			Adenoma	Carcinoma†			
None	M	25	0	0	1	1 (4)§	
	F	24	0	0	0	0	
Trp-P-1	M	24	1	4	0	5 (21)	< .179
	F	26	2	14	0	16 (62)	< .001
Trp-P-2	M	25	1	3	0	4 (16)	< .348
	F	24	0	22 (2)‖	0	22 (92)	< .001

*Number of mice surviving on day 402, when the first hepatic tumor was found. †Mice with both hepatocellular adenoma and hepatocellular carcinoma are included under hepatocellular carcinoma. ‡Statistical significance of the difference in incidence of hepatic tumors between control and Trp-P-1 or Trp-P-2 groups by χ^2 test. §Numbers in parentheses are percentages. ‖Number in parentheses is number of mice with pulmonary metastases of hepatocellular carcinomas.

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