colony that had been propagated for 36 generations in this laboratory and in which "spontaneous" intestinal tumors have not been observed. The rats were maintained in plastic boxes under room conditions (72°F, 70 percent humidity, and a 12-hour light-dark cycle). They were fed sterilized Tek-Lad food (L-485) and provided with ground corncob bedding.

After 20 weeks intestinal tumors were found in 28 of the 39 Sprague-Dawley rats (average, 1.4 tumors per rat) (Table 1). More tumors developed in the small intestine than in the colon. None of the Lobund-Wistar rats developed tumors. Their unique resistance to the carcinogenic effects of DMH and DMN-OAc warrants further study, especially since the latter agent may be a direct carcinogen.

We then injected 30 Sprague-Dawley rats with DMN-OAc and, 14 days later, fed groups of them indomethacin (20 mg/liter) in their drinking water. Fresh indomethacin was provided at 3-day intervals. Control rats were given drug-free water. (It was calculated that the drug-treated rats consumed 3 mg of indomethacin per kilogram per day). All the rats were killed 20 weeks after the injections. In the two trials, the number of indomethacin-treated rats with intestinal tumors was significantly smaller than the number of control rats with tumors (Table 2). The control rats had tumors similar in size, number, and distribution to those described by Joshi *et al.* (*1*) and Berman *et al.* (*2*) and to those observed in the preliminary trials noted above.

It has been determined that DMN-OAc is inactivated within 48 hours after its injection into rats (*5*). Since the indomethacin treatments were started 14 days after the rats were injected with the carcinogen, the antitumor effect may be interpreted less as chemoprevention than as therapy or antipromotion. The results are in agreement with the antitumor effects of indomethacin in rats that were given DMN and MAM (*4, 6*).

Thus, indomethacin has a therapeutic or antipromotional effect against autochthonous intestinal tumors induced by DMH (*4*), MAM (*4, 7*), *N*-methyl-*N*-nitrosourea (*8*), and DMN-OAc. An interpretation of this effect is based on the production of prostaglandins by intestinal tumors (*9*). Agents that block the synthesis of prostaglandins may prevent development of the tumors. However, prostaglandins may not actually have a role in the cytostatic action of anti-inflammatory drugs (*7*).

Indomethacin has retarded the growth of transplanted tumors (*10*). The more precise effect on autochthonous tumors supports the proposition that primary tumors should be used in the assessment of putative anticancer agents. In this respect, indomethacin has been of some therapeutic value in humans with desmoid tumors (*11*). It is important to determine whether the antitumor activity of indomethacin is curative or suppressive, and whether it is effective on advanced intestinal tumors.

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5 May 1981; revised 23 July 1981

Functional Characteristics of the Blood of the Deep-Sea Hydrothermal Vent Brachyuran Crab

Abstract. *Hemocyanin in the whole blood of the hydrothermal vent brachyuran crab,* Bythograea thermydron, *has a moderate oxygen affinity* (P_{50} = *6.6 millimeters of mercury at 2.6°C;* p*H 7.5), which unlike that of other hemocyanins is independent of temperature over the range 2° to 30°C; carbon dioxide and* p*H have independent effects on the oxygen affinity of this pigment. The* p*H effect on affinity is moderate* ($\Delta log\ P_{50}/\Delta pH = -0.34$), *whereas increased carbon dioxide, which can act both directly and by changing* p*H, has a much larger effect* ($\Delta log\ P_{50}/\Delta pH = -0.81$). *This blood has a moderately high degree of cooperativity (Hill cooperativity coefficient,* n, *was 2.8) and a large oxygen-carrying capacity for a crustacean (4.5 milliliters of oxygen per 100 milliliters of blood). These properties characterize an oxygen transport system whose function appears to be largely independent of the wide range of environmental conditions encountered around the vents.*

The brachyuran crab, *Bythograea thermydron*, is an active scavenger around the recently discovered deep-sea hydrothermal vents, at a depth of 2500 m (*1, 2*). Although typically found foraging among the vestimentiferan tube worms that are aggregated close to the vent fissures, the crabs have been observed throughout the vent area (*3*). Because of the rapid mixing of water emerging from the fissures (up to 22°C, anoxic, 350 μm of H_2S, *p*H 6.5) with the surrounding bottom water (2°C, 110 μm of O_2, no H_2S, *p*H 7.5), environmental conditions in the vent area are extremely variable in any one spot and change dramatically within distances of a few centimeters, exposing the crabs to an extreme range of physical and chemical conditions over intervals of the order of minutes (*1, 4*).

We examined the oxygenation characteristics of the whole blood of *B. thermydron* to gain insight into how animals live in this strange environment. We present data indicating that the hemocyanin of the hydrothermal vent crab has oxygenation characteristics that are in some ways unique and that suit this animal to an active life-style in the variable environment of the vents.

The crabs were collected by the submersible *Alvin* during November and December 1979 at the Galápagos Rift Valley sites Garden of Eden, Rose Garden, and Mussel Bed (*5*). Blood samples were taken with a hypodermic syringe from the arthrodial membranes at the bases of the walking legs of live animals that were freshly captured or had been maintained in pressure vessels at 250 atm and 5°C. Absorption spectra were obtained from fresh blood diluted with Millipore-filtered seawater and scanned from 760 to 320 nm. The spectra showed the large peak at 340 nm characteristic of crustacean hemocyanins.

Oxygen equilibrium curves of a small subsample of the fresh whole blood (2 to

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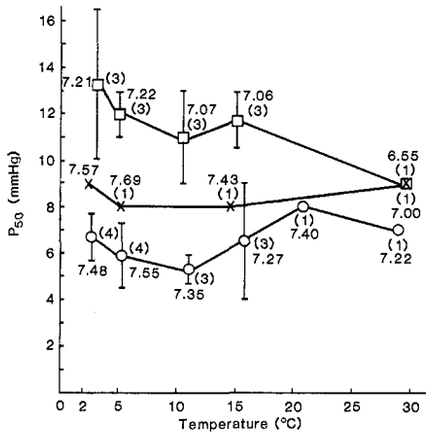

Fig. 1. Oxygen affinity of whole blood as a function of temperature. Data points are means ± 1 S.D.; Measurements at (○) 0.0 percent CO_2 (air), (X) 0.4 percent CO_2, and (□) 2.2 percent CO_2. Numbers in parentheses are numbers of individuals tested, and *p*H values at saturation are given under or over the data points.

4 μl) were measured with a modified Hem-O-Scan (Aminco) at temperatures ranging from 2° to 30°C (Fig. 1) (*6*). The effects of CO_2 were examined at each temperature by generating oxygen equilibrium curves in the presence of 0.03 percent (air), 0.4 percent, and 2.2 percent CO_2. The freshly collected blood sample was maintained in a chamber at the same temperature and CO_2 concentration as the subsample whose oxygen-binding characteristics were being studied. No clotting of the extracted blood was noted. The effect of 0.0 percent (air), 0.4 percent, and 2.2 percent CO_2 on *p*H were determined in this chamber with a combination *p*H probe (E & K) calibrated at *p*H 4 and 7 at each experimental temperature. The 0.4 percent and 2.2 percent CO_2 mixtures were used with 99.99 percent N_2 as the carrier gas, and O_2 and CO_2 content were analyzed by the Haldane manometric method (*7*). Our data were collected at sea from fresh blood samples, except in the one case noted.

The oxygen affinity of the blood of *B. thermydron* showed an unusual independence of changes in temperature. The effect of temperature on the oxygen affinity of decapod crustacean hemocyanins is generally large [ΔH, the enthalpy of oxygenation, varies from −6 to −34 kcal/mole in crustaceans living in shallow waters (*8*)], with increased temperature resulting in greatly reduced affinity. There is, however, no significant effect of temperature on the affinity of *B. thermydron* hemocyanin from 2° to 30°C; the slope of the linear regression line fitting all the data for 0.0 percent CO_2 in Fig. 1 is −0.03 ± 0.6 (95 percent confidence interval; N = 18). The slope of the regression line fitting all the data for 2.2 percent CO_2 in Fig. 1 is 0.13 ± 1.0 (95 percent confidence interval; N = 13). Although effects of temperature on *p*H and on the partial pressure of CO_2 (PCO_2) in vivo may tend to reduce affinity at higher temperatures, the temperature independence of the hemocyanin provides *B. thermydron* with a blood whose oxygenation properties are relatively constant across the wide range of temperatures in the vent environment.

The effect of *p*H on oxygen affinity appears to be paradoxical (Fig. 1). Increased acidity produced by increased CO_2 appears to greatly decrease the O_2 affinity of this hemocyanin. This apparent Bohr shift ($\Delta \log P_{50}/\Delta p$H) from 2° to 15°C has a mean value of −0.81 ± 0.15 (95 percent confidence interval; N = 9) for nine pairs of observations at 0.0 and 2.2 percent CO_2 and at temperatures between 2.5° and 15°C. However, increasing temperature also results in a decline in *p*H at a given PCO_2, although this change does not produce a decrease in O_2 affinity (Fig. 1).

We studied the effect of *p*H by varying it in a series of samples (blood frozen at −85°C for 9 months) with isosmotic HCl and measuring O_2 equilibrium curves at 0.0 percent CO_2 at 4.5°C. These data showed a consistent decline in affinity as *p*H was shifted from 8.1 to 6.5. The value of $\Delta \log P_{50}/\Delta p$H for *p*H change was −0.34 (linear regression coefficient for line fitted to seven data points from *p*H 6.5 to 8.1). The difference between the CO_2 and *p*H Bohr shift values suggests that the hemocyanin of this animal has separate sensitivities to *p*H and CO_2. Another study indicating independent effects of CO_2 and *p*H on crustacean hemocyanins shows comparable effects of CO_2 relative to *p*H (*9*). Separation of the effects of CO_2 and *p*H on affinity could have adaptive value for an animal like *B. thermydron* that is active, yet almost certainly undergoes periods of anaerobiosis. The low *p*H sensitivity could allow blood function to be relatively independent of anaerobic acidosis while retaining, through its CO_2 sensitivity, the facilitation of O_2 unloading at the tissues caused by lowered O_2 affinity there. Oxygen uptake and delivery may also be facilitated in this crab by the moderately high degree of cooperativity of the hemocyanin indicated by the sigmoid shape of the O_2 equilibrium curves. The mean Hill cooperativity coefficient, n, was 2.83 ± 0.18 (95 percent confidence interval; N = 35) (*10*).

Oxygen-carrying capacity is typically low in decapod crustaceans, ranging from 1 to 3 ml of oxygen per 100 ml of blood (*8*). The vent crab, however, has a high carrying capacity of 4.5 ± 0.27 ml of oxygen per 100 ml of blood (95 percent confidence interval; N = 21), determined by a modified Scholander technique (*11*). A high level of energy metabolism, whether supported through aerobic or anaerobic pathways, is not dependent on a high blood oxygen-carrying capacity, as many active crustaceans have low oxygen-carrying capacities (*8*). Thus, the active life-style of the vent crab does not automatically require a high blood oxygen-carrying capacity (*12*). However, this unusual capacity appears to be an important adaptation to hydrothermal vent life, since at least two other vent species have high oxygen-carrying capacities in comparison to their own relatives. The blood of the vestimentiferan tube worm *Riftia pachyptila* has an oxygen-carrying capacity of 8.4 ± 1.8 ml of oxygen per 100 ml of blood [mean ± 1 standard deviation (S.D.), N = 5], and the blood of the vesicomyid clam, *Calyptogena magnifica* carries 4.3 ± 0.92 ml of oxygen per 100 ml of blood (mean ± 1 S.D., N = 3) (*13*). The vent crab, tube worm, and clam are phylogenetically unrelated and have different respiratory pigments; yet each has developed a blood with a substantial capacity to store oxygen, thus suggesting the importance of aerobic metabolism in this heterogeneous environment.

In summary, the oxygen affinity of the whole blood of the vent crab is unusually stable over a wide temperature range, is relatively insensitive to *p*H, and is strongly affected by CO_2. These properties, along with the moderately high degree of cooperativity of the pigment and the ability to carry large amounts of oxygen, characterize an oxygen transport system that is capable of supporting a high level of aerobic metabolism across the entire range of conditions in the Galápagos vent environment without the need for acclimation. These properties suggest that this species can move freely and rapidly throughout its environment without being limited by its oxygen transport abilities.

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6. To establish the accuracy of Hem-O-Scan usage with hemocyanins, we compared data from *Callianassa californiensis* collected with the Hem-O-Scan to previously published results obtained with a different method [K. Miller and K. E. Van Holde, *Biochemistry* **13**, 1669 (1974)]. Our data showed a P_{50} of 8 mmHg at 16°C, *p*H 8.0, $PCO_2 = 0$, and a P_{50} of 14 mmHg at 25°C, *p*H 8.0, $PCO_2 = 0$; $\Delta H = -10.6$ kcal/mole. The Miller and Van Holde data showed a P_{50} of 7 mmHg at 16°C, *p*H 7.8, $PCO_2 = 0$, and a P_{50} of 12 mmHg at 25°C, *p*H 7.8, $PCO_2 = 0$; $\Delta H = -10.2$ kcal/mole. The value of $\Delta \log P_{50}/\Delta p\text{H}$ from the Hem-O-Scan data was calculated to be -1.06, compared to -1.22 with the Miller and Van Holde method. 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 (0.006 mm thick) instead of the copolymer membrane recommended by Aminco for sample containment. In adapting the Hem-O-Scan for use with blood containing hemocyanin, we used interference filters that transmit at 380 and 420 nm. For a more complete description of the Hem-O-Scan see 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. Lapennas, J. M. Colacino, J. Bonaventura, *Methods Enzymol.*, in press.
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27 April 1981; revised 7 July 1981

Dietary Restriction Retards the Age-Associated Loss of Rat Striatal Dopaminergic Receptors

Abstract. *In male Wistar rats subjected to dietary restriction by alternate days of feeding and fasting the normal age-associated loss of striatal dopamine receptors in the brain was substantially retarded. The mean survival time of the rats on the restricted diet was increased by approximately 40 percent compared to control rats given free access to food. Dopamine receptor concentrations in striata of 24-month-old rats that had been on a restricted diet since weaning were 50 percent higher than those of control animals of the same age, and essentially comparable to 3- to 6-month-old control rats.*

An impaired ability to respond and adapt to various stimuli is associated with increasing chronological age (*1*). Such stimuli include drugs, hormones, neurotransmitters, and various physical and chemical agents. The mechanisms responsible for alterations in response to these stimuli include changes in receptors, cell membranes, nuclei and chromatin, cyclic nucleotide metabolism, and various cellular enzyme systems (*2*). Although there have been many attempts to retard the aging process and its associated deterioration, the only confirmed method of life-span extension in mammals to date involves dietary manipulation (*3–5*). It has been suggested that such intervention may act by modifying disease patterns and concomitant destruction of physiological systems (*3–5*).

Table 1. Effect of age and diet on rat striatal dopamine receptor concentrations and affinities. Concentrations (B_{max}) and affinities (dissociation constant, K_D) were determined from individual Scatchard plots, and values are the means ± standard errors for the numbers of experiments indicated. One to three rats were used for each analysis; the number of rats refers to the total number used in the experiments.

Age (months)	Number of		B_{max} (fmole/mg protein)	K_D (n*M*)
	Experiments	Rats		
		Control rats		
3 to 6	8	18	212 ± 12*	13.1 ± 2.4
12	9	20	160 ± 4*	10.3 ± 1.6
24	7	17	132 ± 8*	12.2 ± 3.1
		Rats maintained on restricted diet		
24	7	14	197 ± 8†	15.0 ± 2.4

*Significantly different from other control groups; $P < .01$ (unpaired *t*-test). †Significantly different from 12- and 24-month-old control groups; $P < .01$.

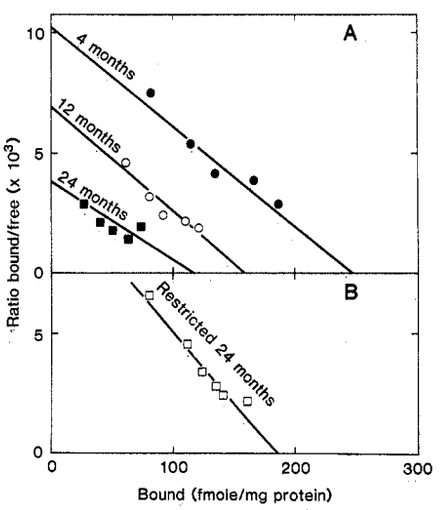

Fig. 1. Scatchard analyses of the binding of ^{3}H-labeled amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to rat striatal membranes. Rat striatal dopamine receptors were measured by the specific binding of 3.5 n*M* to 17 n*M* ^{3}H-labeled ADTN (New England Nuclear, specific radioactivity 35 Ci/mmole) according to the method of Creese and Snyder (*16*), with unlabeled 10^{-5}*M* (+)-butaclamol being used as competitor. Protein concentrations were determined by the method of Lowry *et al.* (*17*) with bovine serum albumin (Pentex) being used as a standard. The animals used were Wistar rats from the colony of the Gerontology Research Center, NIA, Baltimore. The control rats were given free access to NIH or Laboratory Chow; the rats on the restricted diet had access to the same food but only on alternate days from weaning. Body weights were 250 to 500 g, 550 to 650 g, 500 to 600 g, and 350 to 400 g for the 3- to 6-month-old controls, 12-month-old controls, 24-month-old controls, and 24-month-old experimental rats on the restricted diet, respectively. Data were analyzed by the method of Scatchard (*14*) with the use of linear regression analysis for determining slopes of intercepts. (A) Typical Scatchard analysis of control animals aged 4, 12, and 24 months. (B) Typical Scatchard analysis of 24-month-old rats maintained on the restricted diet. Striata from three rats were pooled for each individual analysis.

0036-8075/81/1030-0561$01.00/0