metabolized DDT quite rapidly to DDE. Only small quantities of DDD and the more polar DDA were detected. This is a notable exception to the lack of metabolism seen in the lobster (Toxicol. Appl. Pharmacol. 29:277-288, 1974) and blue crab (Neufeld and Pritchard, unpublished).

In summary, the rock crab gill Na,K-ATPase is sensitive to both in vitro and in vivo exposure to DDT. Since the animal does not osmoregulate, the effect of changing salinities should be minimal although intracellular sodium balance could be disturbed. Additionally, the hepatopancreas concentrates the DDT and metabolizes it to DDE. Depuration appears to occur at a slow rate.

A COMPARATIVE STUDY OF THE RATE OF THE BOHR EFFECT IN VERTEBRATES

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The Bohr effect is the heterotropic interaction of oxygen and hydrogen ions with hemoglobin. The protonation of oxyhemoglobin results in a conformational change in the shape of the molecule, yielding a decrease in the affinity of the heme subunit for oxygen. This increase in the p_{50} with acidosis (or right shift in the O_2 dissociation curve) and oppositely with alkalosis enhances the exchange of oxygen in the tissue and lung capillaries. The rate of this reaction

$$H^+ + HbO_2 \rightarrow HbH^+ + O_2 \uparrow$$

is very rapid, having a half-time of the order of ten milliseconds. Diffusion of carbon dioxide into the red cell followed by its reaction with H_2O provides the change in $[H^+]$. As will be evident, the rate of CO_2 hydration can be a limiting event in the rate of the Bohr effect.

Our interest was aroused by the work of Forster and Steen (J. Physiol. 196:541, 1968), who showed that carbonic anhydrase inhibition produces a thirty-fold reduction in the rate of the overall Bohr effect, suggesting dependency upon the rate of hydration of CO_2. Therefore, the functions of the two major proteins (hemoglobin and carbonic anhydrase) in the red cell are chemically and physiologically linked during capillary transit. Our studies were aimed at further defining the role of carbonic anhydrase in this process with these questions in mind: 1) what is the quantitative relation between carbonic anhydrase inhibition and the rate of the Bohr effect; 2) what (if any) are the physiological and clinical sequelae of a marked reduction in the rate; and 3) is there a relation between the magnitude of the Bohr effect and the carbonic anhydrase activity in the blood of vertebrates. To these ends we studied the rate of the Bohr effect with and without carbonic anhydrase in five different representative vertebrate species: spiny dogfish, *Squalus acanthias;* goosefish, *Lophius americanus;* bullfrog, *Rana catesbeiana;* White Peking duck, *Anas platyrhynchos;* and man.

The experiments were performed following the method of Forster and Steen (vide supra). A 1% suspension of the animal's red blood cells in Ringers solution appropriate for that species was mixed in a rapid reaction apparatus equally with Ringers solution gassed with 20% CO_2 at the animal's body temperature (16°C - 41°C). The increase in pO_2 was monitored by a modified Clark electrode and displayed directly on a chemical Microsensor Model 1201 (Transidyne General Co.). Both solutions were gassed with 2% O_2 except for human blood experiments in which 5% O_2 was used. The carbonic anhydrase inhibitor methazolamide was incubated in both solutions for thirty minutes. The reaction is shown in Figure 1. When the suspension of red cells at < 8 mm Hg pCO_2 mixes with the extracellular buffer solution at 150 mm Hg pCO_2, there is an immediate disequilibrium within the red cells favoring the entry and hydration of CO_2 and formation of acid. This occurs because pCO_2 quickly equilibrates within and outside the red cells, while the relative impermeability of the red cell membrane to H^+ and HCO_3^- limits their movement. Therefore, the initial conditions in the red cell for the hydration of CO_2 are the instantaneously equilibrated pCO_2, and the pH and $[HCO_3^-]$ of the red cell prior to mixing.

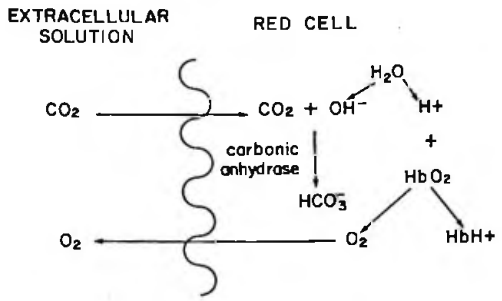

Figure 1. Reactions used to measure the rate of the Bohr effect.

Figure 2 gives the results for the five species. The half-time of the normal Bohr effect in the span of vertebrates studied is quite similar at 120-340 msec. Indeed the differences are likely to be found in the temperature dependence of the hemoglobin protonation reaction. Forster and Steen (vide supra) showed that the half-time of the Bohr effect in human red cells at 37°C was 120 msec (exact agreement with our data) and 190 msec at 23°C. This latter value is only slightly larger than the half-time of the rate in our cold blooded animals at 16°C. When carbonic anhydrase is inhibited by 10^{-3} M methazolamide, the rate of the O_2 release is slowed 15-40 times. The reaction is only 40% complete in man and duck at nine seconds, and 20% complete at this time in fish and frog.

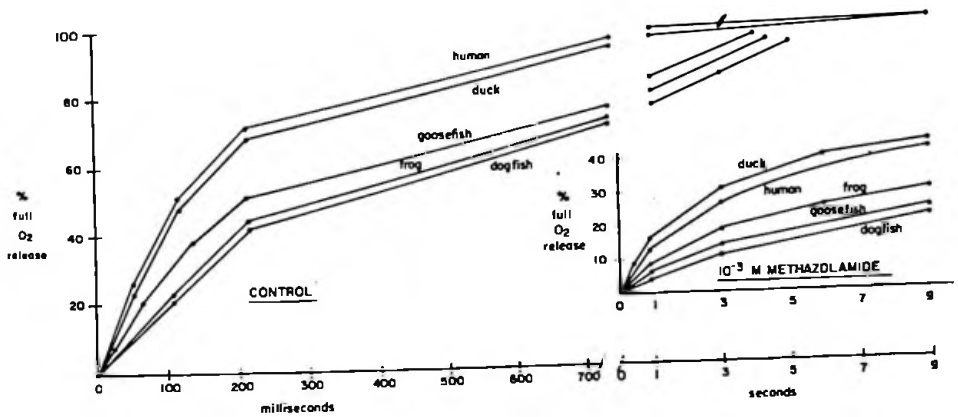

Figure 2. Time course of Bohr effect in normal red cells and following carbonic anhydrase inhibition (inset).

Table 1 gives the chemical rates of hydration of CO_2 in red cells, and the effects of inhibition of carbonic anhydrase; these will be compared to the data of Figure 2. The initial uncatalyzed rate of CO_2 hydration is calculated by the equation

$$V_{uncat} = k_{CO_2} [CO_2] - k_{HCO_3} [HCO_3^-]$$

where k gives the rate constants at the appropriate temperature and brackets show concentration of reactants. The experiment produces a greater than ten-fold increase in the pCO_2 of the cell so that the rate of HCO_3^- dehydration (second term in the equation) is less than 10% of the CO_2 hydration rate. Initial rates of CO_2 hydration (or H^+ production) within the red cell obtain throughout since the slow CO_2 reactions outside the very dilute suspension of cells do not deplete CO_2. Values for initial catalyzed CO_2 hydration (Column 2) are calculated from

TABLE 1

Activity and inhibition of red cell carbonic anhydrases

	V_{uncat} (mM sec^{-1})	V_{cat} (mM sec^{-1})	K_I (nM)	% Residual Activity[1]	$V_{residual\ cat}$[1] (mM sec^{-1})
Human					
Enzyme B	0.160	150	20	0.0020	0.003
Enzyme C		3270	4	0.0004	0.013
Duck	0.210	150	13	0.0014	0.002
Frog	0.057	498	15	0.0015	0.007
Goosefish	0.057	380	10	0.0010	0.004
Dogfish	0.057	182	100	0.0100	0.018

See text for equations and definitions of terms.

[1] At 10^{-3} M methazolamide. $V_{residual\ cat} = V_{cat}\ (1 - i)$.

$$V_{cat} = \frac{k_{cat} \cdot [E_o] \cdot [S]}{K_m + [S]}$$

where k_{cat} and K_m are the kinetic parameters for carbonic anhydrase, E_o and S are the concentrations of enzyme and substrate in the red cells. Column 3 lists the dissociation constants (K_I) of methazolamide for each of the carbonic anhydrases. From these K_I values and the concentration of methazolamide ($I = 10^{-3}$ M) used, the fractional inhibition (i) of the enzyme is calculated, $i = I/(I + K_I)$. This yields the percent residual activity, $(1 - i) \times 100$, which is entered in Column 4. Column 5 gives the residual activity of carbonic anhydrase at 10^{-3} M methazolamide from Col. 2 x Col. 4. Note that residual $V_{cat} < V_{uncat}$.

The data of Table 2 show the relation between observed O_2 release and proton formation from CO_2 hydration. Values for the initial rate of O_2 release (Col. 3 and 5) are taken directly from Figure 2, in which we calculate the rate of O_2 release per unit volume of red cells by the rise in pO_2 of the total suspension. The values for H^+ production in the control experiments are those from the normal activity of carbonic anhydrase as listed in Table 1. It is seen at once that in the normal or control state the catalytic activity of carbonic anhydrase so greatly accelerates CO_2 hydration and H^+ generation, that the latter rate is greatly in excess of observed O_2 release.

When carbonic anhydrase is inhibited, the generation of H^+ becomes rate-limiting, and we can study the relation between O_2 release and H^+ production. This is seen in Table 2, Cols. 4 and 5; the two rates are roughly equal. At 50% O_2 saturation, the Bohr effect factor ($\Delta \log p_{50}/\Delta$ pH) for most vertebrates varies between -0.25 and -0.75 (Hilpert et al. Am. J. Physiol. 205:337, 1963 and Riggs, Chap. 6. Fish Physiology Vol. IV, 1970). This means that for every mmole of H^+ bound, hemoglobin will give up 2 mmoles of O_2. Comparison of the H^+ generation and O_2 release in these species suggest strongly the linkage of H^+ and O_2 as dictated by the Bohr effect factor, when the formation of H^+ is the rate-limiting step. For man, whose Bohr effect factor equals -0.48 and where data on hemoglobin and carbonic anhydrase are most extensive, O_2 release and proton formation are linked exactly 2:1. Despite the wide differences in red cell carbonic anhydrase activity among the vertebrates, the catalysis is always far greater than necessary (Compare Cols. 2 and 3 of Table 2) and bears no relation to the magnitude of the Bohr effect. This is similar to all other systems involving this enzyme.

TABLE 2

Rates for the Bohr effect in five vertebrates compared with the chemistry of H^+ generation

	Bohr Effect Factor $\Delta \log p_{50}/\Delta$ pH	Control		10^{-3} M Methazolamide	
		H^+ Gen.	O_2 Release	H^+ Gen.	O_2 Release
		millimoles per liter · sec^{-1}			
Frog	-0.25	498	0.55	0.064	0.053
Dogfish	-0.34	182	5.0	0.075	0.070
Goosefish	-	380	18.0	0.064	0.17
Duck	-0.53	150	13.0	0.212	0.19
Human	-0.48	3420	16.5	0.176	0.38

Frog, dogfish and goosefish blood were studied at 16°C, duck at 41°C and human at 37°C. The values in Column 2 are from the calculated V_{cat} of Table 1. Columns 3 and 5 from the observed data of Figure 2. The values in Column 4 are the sum of the uncatalyzed and residual catalyzed rates given in Table 1.

The rate of the Bohr effect is so diminished at 10^{-3} M methazolamide that the amount of oxygen exchange thus mediated cannot occur within capillary transit time (inset, Figure 2). At lesser concentrations of methazolamide, 10^{-4} and 10^{-5} M, the rate of the Bohr effect is still slowed, precluding the majority of its contribution in oxygen transfer (Swenson and Maren, Bull. MDIBL Vol. 16, 1976). These latter levels are readily achieved in vivo and yet no overt toxicity has been reported. The organism may compensate by increasing the blood flow, dilating and recruiting capillaries, increasing red cell 2,3-diphosphoglycerate, widening pO_2 and pCO_2 AV gradients or shifting to a greater degree of anaerobic metabolism. Whether this imposes any physiological disadvantage, especially in conditions of cardiovascular or pulmonary compromise, remains for further investigation.

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PERMSELECTIVE PROPERTIES OF THE SECRETORY EPITHELIAL BARRIER IN THE ISOLATED PERFUSED RECTAL GLAND OF THE SPINY DOGFISH (*Squalus acanthias L.*)

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The constraints upon passive fluxes of metabolically-inert, lipid-insoluble electrolytes across secretory epithelial barriers are difficult to evaluate in vivo owing to a complex interplay of local fixed or induced charges, potential differences generated during active transport of ions, Donnan effects, unstirred layer artefacts and diffusion potentials. Direct measurement of transmembrane potential gradients may be impossible in many tissues in which membrane surfaces are inaccessible, as in the hepatic canaliculi, or where introduction of micro-electrodes may produce functional disturbances that obscure interpretation.

A means of surmounting this difficulty has been suggested by the observation (Bradley and Herz, Diseases of the Liver and Biliary Tract, 5th Quatr. Meeting Int. Assoc. Study of the Liver, Acapulco 1974, pp. 113-116, Karger, Basel, 1976) that the biliary ^{14}C-ferrocyanide clearance (C_F) in rats is considerably less than the simultaneously determined biliary ^{3}H-sucrose clearance (C_S) despite similar molecular weights