kidney wet weight, the $T_{max}$ would then be 1694 (50% tubules) to 3388 (25% tubules) nmol/hr/g tubules. These values are quite close to the $V_{max}$ predicted by the kinetic experiments discussed above, 3900 nmol/hr/g tubules.

Finally, we tested the effectiveness of 2,4-D as an inhibitor of PAH transport in vivo. It was inhibitory, producing 75% inhibition at a dose of 25 µmol/kg (10 x the substrate dose), much less than CPR, PROB, or DDA (Bull. MDIBL 16:55-58, 1976). This result was predicted by the kinetic studies since the $K_i$ for 2,4-D (44 µM) was much higher than the $K_i$ for the others (5-15 µM).

In conclusion, 2,4-D is actively transported on the renal organic acid system in fish as it is in mammals. Its plasma binding is less extensive than DDA and its renal transport is consequently more effective in vitro and in vivo, reflecting its greater availability at the transport site. Its transport in vitro and in vivo is inhibited by other organic acids, and it is an inhibitor of transport of other organic acids. However, due to its lower affinity for the carrier, it is a less effective inhibitor than CPR, PROB, or DDA. In vivo 2,4-D shows a $T_{max}$ of ∿ 1000 nmol/hr/g kidney, a value which correlates well with the predictions of in vitro kinetic measurements.

## DDT IN THE ROCK CRAB: TISSUE DISTRIBUTION, METABOLISM AND INHIBITION OF GILL Na,K-ATPase

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The Na,K-ATPase of many aquatic organisms has been shown to be sensitive to DDT inhibition in vitro (Nature 233:148-149, 1971; Comp. Biochem. Physiol. 40B:823-827, 1971). However, in only a few studies with teleosts has it been possible to show both Na,K-ATPase inhibition and osmoregulatory deficits following in vivo exposure to DDT (Environ. Health Perspect. 1:169-173, 1972; Comp. Biochem. Physiol. 49A: 197-205, 1974). While several crustaceans were shown to be highly sensitive to organochlorine pecticides, sublethal doses had little effect upon their ability to hyperosmoregulate in a dilute environment (Environ. Pollut. 8:283-300, 1975; Pollution and Physiology of Marine Organisms, F. John Vernberg and Winona B. Vernberg, Eds. Acad. Press, 1974, pp. 427-443).

The objectives of this study were to determine the in vitro and in vivo effects of DDT on gill Na,K-ATPase from the rock crab, *Cancer irroratus*. Secondary objectives were to determine the tissue distribution and metabolism of DDT after intravascular injection.

Hemolymph samples were obtained by puncturing the membrane at the base of one of the walking legs; osmolarity was then measured by vapor pressure osmometry (Wescor, Inc.). Na,K-ATPase was assayed essentially as described by Miller et al. (Amer. J. Physiol. 231:370-376, 1976). The final assay medium contained the following concentrations: 50 mM NaCl, 10 mM KCl, 4 mM $MgCl_2$, 2 mM disodium ATP, 0.67 mM EDTA, 92 mM Tris (pH 7.4). The distribution and metabolism of $^{14}$C-DDT were determined as described by Guarino et al. (Toxicol. Appl. Pharmacol. 29:277-288, 1974).

Although many of the decapod crustacea hyperosmoregulate very effectively in water of low salinity, the results shown in Figure 1 illustrate that *Cancer irroratus* osmoconforms over the range tested. Furthermore, there was no significant increase in gill Na,K-ATPase activity when crabs were adapted to 50% seawater (7-10 days) as occurs in hyperosmoregulators such as the blue crab (Amer. Zool. 16:223, 1976; J. Exp. Zool. 196:315-321, 1976). The specific activity of the enzyme remained about 2.7 µmoles $P_i$/mg protein/hr when assayed at 30°C. These data do not agree with those of Cantelmo et al. (Comp. Biochem. Physiol. 51A:537-542, 1975), who concluded that the rock crab was able to hyperosmoregulate in seawater below 75%.

Both in vitro and in vivo exposure of the gill Na,K-ATPase to DDT resulted in significant inhibition of the enzyme (Figures 2 and 3). DDT at 0.5 ppm (1.43 µM) produced significant inhibition in vitro with

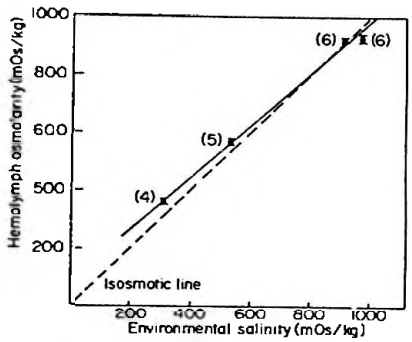


Figure 1. Rock crab hemolymph osmolarity at various seawater salinities. The numbers in parentheses represent the sample sizes. Males and females were grouped together since there were no significant differences between their osmolarity values.

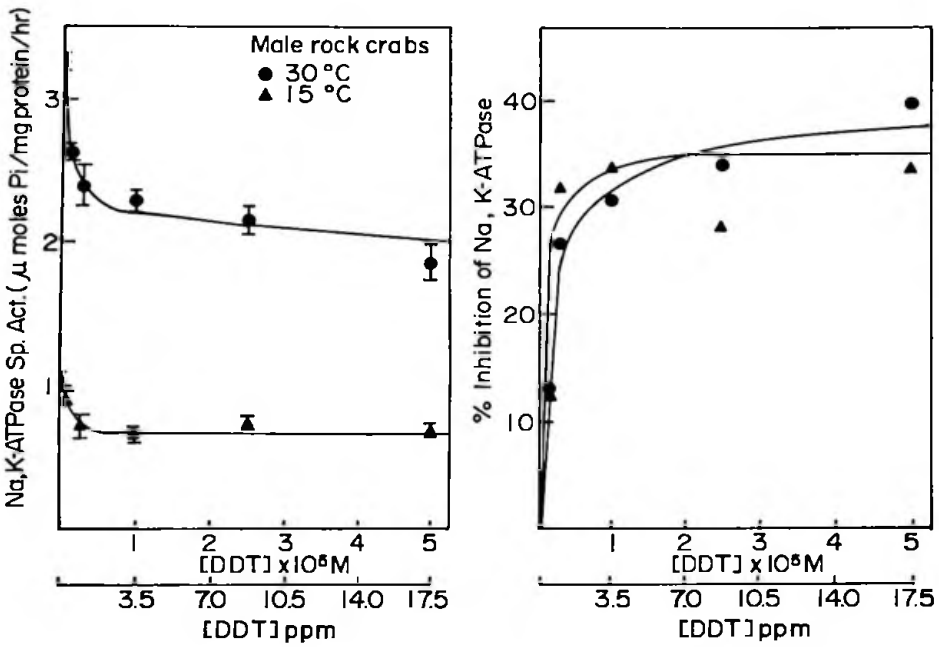


Figure 2. Inhibition of male rock crab gill Na,K-ATPase in vitro at various DDT concentrations. Each point represents the mean ± SE of five separate determinations.

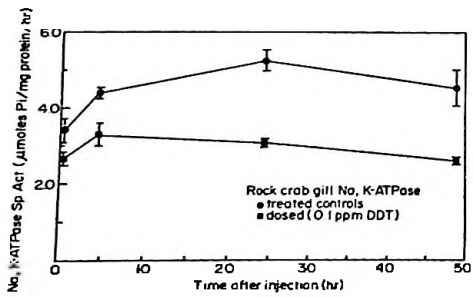


Figure 3. Effect of DDT male rock crab gill Na,K-ATPase after in vivo exposure by intravascular injection. The enzyme was assayed at 30°C. Each point is the mean ± SE of values from four individual animals.

maximal inhibition at 3-4 ppm. Furthermore, there was no significant difference in DDT sensitivity when the enzyme was assayed at 30°C or at 15°C, the temperature of the bay water from which the animals were taken. The DDT sensitivity of the rock crab Na,K-ATPase was very similar to blue crab Na,K-ATPase assayed under comparable conditions (Environ. Health Perspect. 1977, In press).

When the rock crabs were exposed to 0.1 ppm DDT via intravascular injection, the gill Na,K-ATPase again was inhibited (Figure 3). Since the rock crab does not osmoregulate, it should not affect hemolymph sodium although it could alter the intracellular sodium balance.

To assess the quantity of DDT reaching the gills in the in vivo studies above, 0.1 ppm $^{14}$C-DDT was given intravascularly. As shown previously in lobster and shrimp (Toxicol. Appl. Pharmacol. 29:277-288, 1974; Bull. Environ. Contam. Toxicol. 5:333-341, 1970), the hepatopancreas rapidly accumulates the bulk of the labeled DDT (Table 1). The maximum concentration was reached in the hepatopancreas by 48 hrs and decreased very little by 21 days post-treatment. The other tissues exhibited maximum values much earlier and then decreased until a steady state was achieved. In particular, the gills reached a value of 0.3 µg/gm by 1 hr, fell to 0.1 g/gm by 24 hrs, and decreased very slowly thereafter. Table 2 shows that the rock crab

TABLE 1. TISSUE DISTRIBUTION OF RADIOLABELLED COMPOUNDS AFTER INTRAVASCULAR ADMINISTRATION OF $^{14}$C-DDT*

| | Amount (µg/gm tissue) | | | | | |
|---|---|---|---|---|---|---|
| Tissue | 1 hr | 4 hr | 24 hr | 48 hr | 7 days | 21 days |
| Blood | 0.110 ± 0.018 | 0.043 ± 0.004 | 0.028 ± 0.004 | 0.020 ± 0.001 | 0.028 ± 0.008 | ----- |
| Hepatopancreas | 0.200 ± 0.079 | 0.505 ± 0.048 | 1.304 ± 0.188 | 1.650 ± 0.163 | 1.653 ± 0.123 | 1.406 ± 0.135 |
| Heart | 0.467 ± 0.057 | 0.213 ± 0.027 | 0.132 ± 0.020 | 0.062 ± 0.009 | 0.051 ± 0.010 | 0.045 ± 0.003 |
| Stomach | 0.112 ± 0.004 | 0.104 ± 0.007 | 0.062 ± 0.013 | 0.053 ± 0.008 | 0.053 ± 0.013 | 0.040 ± 0.003 |
| Intestine | 0.299 ± 0.075 | 0.220 ± 0.033 | 0.132 ± 0.013 | 0.131 ± 0.022 | 0.097 ± 0.021 | 0.112 ± 0.022 |
| Gonad | 0.133 ± 0.012 | 0.164 ± 0.019 | 0.148 ± 0.021 | 0.082 ± 0.002 | 0.063 ± 0.008 | 0.067 ± 0.005 |
| Gill | 0.304 ± 0.048 | 0.132 ± 0.012 | 0.089 ± 0.019 | 0.056 ± 0.001 | 0.059 ± 0.015 | 0.069 ± 0.003 |
| Muscle | 0.111 ± 0.011 | 0.128 ± 0.009 | 0.082 ± 0.013 | 0.044 ± 0.003 | 0.025 ± 0.006 | 0.033 ± 0.002 |
| Epidermal membrane | 0.136 ± 0.027 | 0.306 ± 0.015 | 0.170 ± 0.007 | 0.165 ± 0.020 | 0.128 ± 0.023 | 0.161 ± 0.030 |
| Green gland | 0.239 ± 0.063 | 0.291 ± 0.012 | 0.141 ± 0.026 | 0.098 ± 0.009 | 0.068 ± 0.005 | 0.080 ± 0.007 |
| Brain | 0.394 ± 0.018 | 0.337 ± 0.031 | 0.150 ± 0.14 | 0.111 ± 0.030 | 0.115 ± 0.011 | 0.182 ± 0.060 |

*The $^{14}$C-DDT was administered at a dose of 0.1 mg/kg into the pericardial sinus in 10% ethanol, 10% Emulphor, 80% saline. All values are given as mean ± SE for 3-4 animals at each time period.

TABLE 2. THIN-LAYER CHROMATOGRAPHY ANALYSIS FOR DDT AND METABOLITES IN ROCK CRAB HEPATOPANCREAS

| | % of Total Radioactivity* | | | |
|---|---|---|---|---|
| Time after injection | DDT | DDD | DDE | DDA |
| 4 hr | 85.3 ± 0.8 | 6.3 ± 0.8 | 4.8 ± 0.6 | 3.7 ± 0.8 |
| 24 hr | 72.7 ± 5.4 | 7.2 ± 1.8 | 17.3 ± 3.8 | 2.6 ± 0.4 |
| 48 hr | 56.9 ± 3.9 | 18.3 ± 2.5 | 21.8 ± 2.0 | 3.0 ± 0.8 |
| 7 days | 46.3 ± 6.1 | 6.3 ± 1.5 | 43.9 ± 4.7 | 3.6 ± 0.3 |
| 21 days | 7.7 ± 3.2 | 6.7 ± 0.9 | 80.1 ± 4.2 | 4.8 ± 0.3 |

*Each value is the mean ± SE.

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.