TABLE: Comparison of Carbonic Anhydrase Kinetics Between Dog and Dogfish Blood*

Temp.	Species†	V_{max} μmol/liter per sec	V_{max}/E = Turnover number : min^{-1}	K_m mM
5°	S	350	3×10^6	12
	D	1000	16×10^6	23
16°	S	510	4.4×10^6	12
	D	1400	22×10^6	26
37°	S	1416	12×10^6	50
	D	2500	40×10^6	50

*All experiments were done in barbital buffer system, using 0.1 ml of 1:10 dogfish blood diluted with water. This amount of enzyme is equivalent to 1 enzyme unit when tested at 5° and 100% CO_2. The molar equivalent (E) for 1 unit is 7×10^{-9}M.

†S = S. acanthias blood.
D = Dog blood data taken from J. Pharm. Expt. Therap. 130: 129, 1963. For 5° and 16° extrapolated figures are used from original data at 0°, 12°, and 24°. In these experiments three enzyme units were used, equivalent to 3.7×10^{-9}M.

ences in turnover number—that for dog blood being in the range of fourfold higher at each temperature. K_m values are roughly the same. These data support the finding given in another report for 1965, that sulfanilamide yields a very different K_i for the two enzymes.

Supported by NIH grant NB-01297.

1965 #26

CARBONIC ANHYDRASE ACTIVITY AND INHIBITION IN TISSUES OF FISH AND AMPHIBIA

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I. Enzyme Activity. It was desirable to extend and re-evaluate certain data of 1958 (Bull. Mt. Desert Island Biol. Lab. IV, part 3, p. 72) in which the carbonic anhydrase activity of submammalian species was studied. As will be evident, use of a different buffer system in the assay had in certain cases an important and unexpected result. Methods were those described in J. Pharm. Exptl. Therap. 130: 389, 1960. Blood contamination of the organs was minimized by bleeding the animal before dissection, and washing the tissue in 0.25 M sucrose in tris buffer. Observations of particular physiological relevance follow:

Kidney. Table 1 shows that in most tissues of Squalus acanthias, the carbonate buffer system yields much lower results than barbital. Presumably, carbonate is inhibitory to the enzyme. In the barbital system, renal carbonic anhydrase is detectable, while in carbonate it is absent or equivocal. Since neither marine elasmobranch nor teleosts can alkalinize their urine in response to large doses of carbonic anhydrase inhibitors, the role of renal enzyme in these teleosts has been a mystery for some years, and now the same problem presents for the elasmobranch. At the suggestion of Dr. Leon Goldstein, we looked for enzyme in different regions of

Table 1

CARBONIC ANHYDRASE ACTIVITY (ENZYME UNITS/g WET WEIGHT) IN TISSUES OF FISH AND AMPHIBIA

Species	Blood	Kidney	Rectal gland	Pancreas	Stomach	Liver
S. acanthias	100	12	204	13	-	9
	7	0-3	133	0	40	-
M. octodecimspinosus	295	110				
	18	15				
M. scorpius	760	250*				
	-	-				
Gadus callarias	1138					
	395					
Protopterus aethiopicus	-					
	14					
Xenopus laevis	-	-				
	80	37				
Rana catesbiana					500†	
					114	

The first row for each species gives data for the barbital buffer system, the second for carbonate.

*Tissue from caudal, middle and cephalad portion all had about the same enzyme concentration. There was no renal tissue in the cephalad portion, but tubules were readily visualized in the caudal part. For this observation we are indebted to Leon Goldstein and Roy Forster.

†Epithelium, dissected by Adrian Hogben in the manner used for in vitro secretory studies. The units are equivalent to a molar concentration of enzyme of 7.6×10^{-6}.

the kidney of the short horn sculpin M. scorpius. As noted in Table 1, enzyme was present in high concentrations, as found also for other teleosts (1958 data cited above). However, the cephalad portion, which contains no renal but much hematopoietic tissue had as much enzyme as the true excretory tissue. From these and the physiological results, we suppose that the carbonic anhydrase in kidneys of marine fish has nothing to do with renal function, but is part of the blood forming system. The same explanation may apply to Xenopus laevis, since Dr. Leon Goldstein cites work from his laboratory (by G. M. Fanelli and R. L. McBean) in which acetazolamide failed to alkalinize the urine of this species.

Pancreas and Liver. The new finding of carbonic anhydrase in these organs of fish suggests that their HCO_3^- accumulating function, which has been well studied in the mammal, is a fairly stable vertebrate pattern.

Rectal Gland. This confirms our earlier work and lends emphasis to the current studies of R. Palmer in which he inhibited rectal gland secretion in vitro by carbonic anhydrase inhibitors.

Stomach. This was carried out in connection with Dr. Adrian Hogben's secretory work. The enzyme unit activity was followed up by a kinetic analysis of the inhibition by a powerful sulfon-

amide, which yields the molar concentration of enzyme, in the manner of the 1960 paper cited above. This value is approximately 8 μM, and since about 1/4 the volume of the epithelial strip is reckoned as parietal cell, the carbonic anhydrase concentration of the active tissue is taken as 30 μM (Frog).

Blood. Table 1 includes some data for species not heretofore examined; which supports the idea that all vertebrates probably have carbonic anhydrase in their red cells. Several liters of blood from S. acanthias was collected for separation and chemical characterization of carbonic anhydrase. Results will be reported separately, along with a comparison to mammalian enzyme.

II. Inhibition. Six carbonic anhydrase inhibitors of varying potency, structure, and physical and chemical properties were studied against many of the enzyme sources of Table 1. This has two purposes: a) Some of these drugs have been used in physiological experiments, so their potency against the enzyme in the organ studied was of cardinal importance. For example, the failure of acetazolamide to alkalinize the urine of marine fish could conceivably have been due to a refractory enzyme in fish kidney—such an enzyme has been found in plants and in rat liver. b) The inhibition potency of these drugs against the various enzymes from different sources (recorded here as the I_{50} or molar concentration to inhibit 50% of enzyme in vitro) provides a sensitive although qualitative index as to whether these are in fact different or the same proteins.

The six drugs, together with their $I_{50} \times 10^{7}$ M against pure human red cell carbonic anhydrase fraction C, follow: Sulfanilamide (24); acetazolamide (0.3); methazolamide (0.2); CL 11,366 (0.04); CL 13,580 (0.04); ethoxzolamide (0.04). The structures and properties of these drugs are given in Acta Pharm. et Tox. 17: 315, 1960 and J. Pharm. and Exptl. Therap. 139: 140, 1963. Except for sulfanilamide, the drugs gave I_{50}'s roughly comparable (within 7-fold) to the above when tested against the enzyme sources of Table 1. The larger of these variations were attended by some uncertainty in the assay, due to the low concentration of enzyme present. "Complete" inhibition was achieved by increasing the I_{50} about 10-fold. Point a) above is thus answered, in that physiological experiments with, for example acetazolamide, can be expected to inhibit the enzyme in these species. Inhibition kinetics in vivo will not be greatly different from those described for the dog in the 1963 paper cited above. With respect to point b) the data for sultanilamide suggest that some of these enzymes are quite different chemically from each other and from mammalian carbonic anhydrase. For example dogfish blood had an I_{50} of 872×10^{-7} M and rectal gland of 164×10^{-7}M. Sculpin blood was 116×10^{-7} M. It is of interest that the weakest inhibitor was the one to show the most clear variations.

In connection with the idea broached in part I, that the kidney enzyme in these species is actually part of the hematopoietic system, it was of interest that I_{50}'s for any given drug against blood and kidney of the same species was always the same.

Supported by NIH Grant NB-01297.