Fish	Q_{O_2} (means of two fish)	
	Actual	Relative (total, per unit body weight)
Eel (Anguilla rostrata)	3.1	1.0
Pollack (Pollachius virens)	1.5	2.2
Sculpin (1 fish) (Myoxocephalus scorpius)	4.3	1.7
Skate (Raja ocellata)	1.4	2.3
Dogfish (Squalus acanthias)	0.9	3.5

probably the major site of GDH activity in this species. The kidneys also appear to be important, contributing up to 20% of the total ammonia produced by GDH, and might possibly retain this role in salt water forms in which urine production is curtailed.

From the preceding observations it can be calculated that the GDH in the liver and kidneys of a 100 gram eel could produce, at physiological glutamate concentrations (ca. 10 mM) and under optimal assay conditions, 95 μ-moles of ammonia per hour. By comparison, two eels studied in vivo excreted an average of 16 μ-moles/100 g-hr. Therefore the in vitro glutamic acid dehydrogenase activity of liver and kidney can more than account for the ammonia excreted by the eel.

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1965 #25

TEMPERATURE OPTIMA AND KINETIC DATA FOR CARBONIC ANHYDRASE IN COLD AND WARM BLOODED VERTEBRATES

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The V_{max} and turnover number of carbonic anhydrase from dog red cells increases three-fold as temperature is raised from 0° to 37°C. Over this range the Km increases two-fold. It is of interest that over the same temperature range the uncatalyzed hydration of CO_2 increases seventeen-fold (J. Pharm. Exptl. Therap. 139: 129, 1963).

Similar studies were carried out on carbonic anhydrase from dogfish red cells. The Table (p. 26) gives the data along with previously published data for dog red cell enzyme. Each figure is the mean from 2-4 plots of $1/V$ against $1/(CO_2)$ with V_{max} read as the ordinal intercept and K_m taken as the substrate concentration which yielded a rate $1/2V_{max}$. The value for E, the molar concentration of enzyme present, was obtained by what is essentially a titration against a powerful inhibitor, in this case CL 13,580,2-o-chlorphenylthiadiazole-5-sulfonamide (see J. Pharm. Exptl. Therap. 130: 389, 1960).

The turnover number, which is the critical measure of enzyme activity, increases progressively for the dogfish enzyme from 5° to 37°C, despite the fact that the animal lives at about 16°C in the summer. The quantitative effect of temperature on enzyme activity is precisely the same for the dogfish red cells as for those of the dog.

The data suggest some difference between the two enzyme sources, since the molar equivalent (E) for one enzyme unit of activity is greater for the dogfish. This is reflected in the differ-

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.

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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