green and more modestly to 20 min with 2×10^{-2} M p-aminohippurate or Diodrast.

In these experiments efflux clearly involved movement out of tubular fluid. Thus, the biphasic action of competitors is interpreted as strong evidence for a "carrier" mediated efflux process (introduction). Future efflux studies should be undertaken to determine whether countertransport (exchange) is involved and to explain the unusual Q_{10}.

1965 #24

GLUTAMIC ACID DEHYDROGENASE IN THE EEL (Anguilla rostrata)

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Ammonia is the predominant nitrogenous excretory product of teleost fishes. The enzyme glutamic acid dehydrogenase (GDH) catalyzes the oxidative deamination of glutamate, thereby forming ammonia and α-ketoglutarate. In order to examine the possible importance of GDH to ammonia production by fish, the kinetic properties of this enzyme in eel liver homogenates were examined and the content of the enzyme in several tissues of the eel and in the livers of some other teleost and elasmobranch species was assayed.

The activity of the enzyme was estimated by measuring the oxygen consumption of a tissue homogenate at 30°C with a Gilson differential respirometer; results were expressed as Q_{O_2} on a wet-weight basis, i.e., ml oxygen consumed at S.T.P. per gram wet tissue per hour. Using livers from eels caught in fresh water, and with a tissue concentration of 17 mg/ml, the following final concentrations of constituents of the incubation medium gave maximal oxygen consumption: K glutamate, 0.1 M; H^+, pH = 7.2; DPN, 3.0×10^{-3}M; cytochrome C, 2.7×10^{-5}M; semicarbazide, 0.1 M. There was a stoichiometric relation between oxygen consumption and ammonia production.

By varying glutamate concentration, the maximum reaction rate (V_{max}) was calculated to be 3.5 (Q_{O_2}) and the K_m 1.6×10^{-2}. Ammonia inhibited the reaction "competitively", with a K_i of approximately 1×10^{-2}. In the presence of 0.05 M NH_4Cl, the deamination rate was reduced by about 40% at a glutamate concentration of 0.1 M and by 55% at a glutamate concentration of 0.01 M.

When glutamate was replaced by alanine (0.1 M) and semicarbazide omitted, the Q_{O_2} was 0.3 or about 16% of the rate of oxidation of glutamate without semicarbazide. Semicarbazide reduced the Q_{O_2} (alanine) by one-half. In the absence of semicarbazide and in the presence of added pyridoxal phosphate (10^{-4}M) and α-ketoglutarate (10^{-3}M), the Q_{O_2} was 0.7 or 37% of the rate with glutamate. These results indicate that, in this system, alanine is oxidized by transamination with α-ketoglutarate to form glutamate, which is then oxidized by GDH.

The GDH activities in the livers of some marine species are compared to the activity of eel liver in the table on the following page.

While these data suggest that the more active fish (pollack, dogfish) have a greater relative "content" of GDH, they do not justify a firm conclusion.

Kidney tissue from two eels showed about 50% of the activity found in liver; the gills were low in activity (about 19%), while the activity of muscle was negligible. Liver represents about 0.8% of the body weight of an eel, and kidney and gill tissue about 0.3% each. Thus the liver is

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-