radioautography (11;12). In addition, by applying standard cytochemical techniques at the e/m level to the placental barrier of the dogfish during such visualized transport, it will also be possible to correlate localization of specific enzymatic activities with mechanisms of transplacental exchange.

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1965 #23

MICROSPECTROPHOTOMETRIC STUDY OF CHLORPHENOL RED TRANSPORT IN ISOLATED RENAL TUBULES OF THE FLOUNDER[*]

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In two recent studies employing *in vitro* techniques the efflux (run-out) of pre-accumulated organic anions (Diodrast and p-aminohippurate) from renal tissue (goldfish and dog) was accelerated by the presence of low concentrations of competitors in the efflux medium and decelerated by higher concentrations of the same competitors (Kinter and Cline, *Amer. J. Physiol.*, 201: 309, 1961 and Farah, *et al.*, J. Pharm. and Exptl. Therap., 139: 120, 1964). If it were certain that the pre-accumulated anions were leaving from tubular fluid, i.e., not being displaced from intracellular binding sites, then these biphasic effects would constitute strong evidence that the efflux process was more complex than simple diffusion and involved a "carrier" of the sort generally proposed for the uphill transport producing accumulation. To clarify this point, direct photometri measurement of anionic dye efflux from luminal fluid was undertaken with flounder tubules.

The original isolated flounder tubule preparation was developed by Forster (*Science*, 108: 65 1948). Luminal concentration of anionic dye, initially phenol red and later chlorphenol red, was estimated by eye (zero to 4+) using a standard microscope. With this method Hong and Forster

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made semi-quantitative observations on run-out (efflux) of chlorphenol red from luminal fluid (J. Cell. and Comp. Physiol., 51: 241, 1958). In the present study renal tubules isolated from freshly captured flounder were supported on glass wool within a small optical chamber through which Forster's saline medium was perfused. A recording microspectrophotometer with a 4-8 μ diameter beam of 575 mμ light (absorption peak of chlorphenol red) was used to measure, either continuously or intermittently, the dye concentration in one or more individual tubular lumens (diameters 15-30 μ). During initial perfusion with oxygenated medium containing a low concentration of chlorphenol red (10^{-6} to 5×10^{-5} M), uphill transport into tubular fluid (influx) was rapid, and within 30 min the dye concentrations in many individual lumens had reached high steady state values which ranged up to 4×10^{-2} M at room temperature (22 ± 3C). With influx medium chilled to the approximate temperature of local sea water (12 ± 2C), the steady state values were lower. Dye was never detected in the tubular cells.

Anionic dye influx in isolated tubules is well known to be a function of the uphill transport process responsible for tubular secretion of organic anions in intact kidneys. When studied with the present technique, the initial velocity of chlorphenol red influx exhibited the following four characteristics typical of uphill transport processes in general: saturation, competition, energy dependence, and temperature sensitivity. For example, the dye concentration in influx medium which yielded one-half the maximal value for influx velocity (Km' of Michaelis-Menton kinetics) was 1.1×10^{-5} M at 22C. The average value for the maximal velocity (V_{max}) was 6×10^{-12} μmoles of chlorphenol red per min for each μ^2 of area at the surface of the tubular lumen. Colorless anions, p-aminohippurate and probenecid, gave evidence of inhibiting the velocity of dye influx in a competitive manner (convering lines in a Lineweaver-Burk plot). The following Ki' values were obtained for p-aminohippurate and probenecid, respectively: 4.5×10^{-5} and 7.2×10^{-5} M at 22C. Dye influx stopped when oxidative metabolism was blocked, i.e., V_{max} was not detectably greater than zero when influx medium was equilibrated with N_2 (less than 1% O_2) or contained 0.5 - 1.0 mM cyanide. Finally, lowering the temperature of influx medium and, consequently, the tubules by 10 degrees (from 22 to 12C) decreased influx velocity by more than 50% (Q_{10} averaged 2.3).

Chlorphenol red efflux was measured after a sudden switch to perfusion with each of the following four types of efflux medium, none of which would support influx: (1) O_2 and (2) N_2 equilibrated medium with zero dye; (3) cyanide containing and (4) N_2 equilibrated medium with up to 10^{-4} M dye. Within 2 min of switching, the concentration of dye in luminal fluid began to fall in a strictly exponential manner (plot of log concentration against time gave a straight line for each tubule) and after about 30 min half of the dye had disappeared from the lumen. No detectable concentration of dye (10^{-4} M or greater) ever appeared in the tubular cells. Results were similar with all four types of efflux medium; pooling these "control" results, the mean half-time for chlorphenol red efflux was 29 (SD ± 6) min for 39 tubules at 22C. Surprisingly, reducing the temperature to 12C increased the rate of dye efflux (Q_{10} for half-time averaged 0.34). Finally, addition of known competitors to efflux mediums (1) and (2) was investigated at 22C. Four organic anions were tested, each in a series of experiments which revealed the expected biphasic effect as competitor concentration was raised from 10^{-6} to about 10^{-2} M in succeeding experiments: first mean half-time for chlorphenol red efflux decreased to minimal values, 15 min with 10^{-4} M probenecid or bromcresol green and 8 min with 10^{-3} M p-aminohippurate or Diodrast; and second half-time increased above the control value to 50 min with 10^{-2} M probenecid or bromcresol green and more modestly to 20 min with 2 x 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 x 10^{-3}M; cytochrome C, 2.7 x 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 x 10^{-2}. Ammonia inhibited the reaction "competitively", with a K_i of approximately 1 x 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