Table 2.--Methylamine secretion by isolated perfused renal tubules

	Self-inhibition and effect of methazolamide		
	Control	10 mM methylamine	0.1 mM methazolamide
tubule length, mm	1.061 ± 0.162	0.715 ± 0.130	0.930 ± 0.078
V_c, nl · min^{-1} · mm^{-1}	6.78 ± 0.79	7.16 ± 0.79	6.33 ± 0.62
J, pmole · min^{-1} · mm^{-1}	0.76 ± 0.08	0.47 ± 0.10	0.57 ± 0.05
(P/B)	2.87 ± 0.32	1.24 ± 0.11	1.85 ± 0.13
N;n	5;22	4/14	4/26

Abbreviations: V_c, collection rate; J, excretory flux; (P/B), final perfusate to bath concentration ratio.

Values shown are MEAN ± SE for (n) number of measurements on a total of (N) number of tubules. 50 μM (^{14}C) methylamine was present in the bath and initial perfusate.

DISCUSSION

Results presented here establish that the flounder kidney secretes MA against a concentration gradient by a saturable mechanism. Hence, MA cannot be used as an indicator of intracellular pH in the kidney.

To minimize backflux of MA from lumen to bath, tubules were perfused at the highest rates possible without causing damage to the epithelium. That backflux did occur, and that the epithelium is highly permeable to MA, is suggested by: 1. The final perfusate to bath concentration ratio was the same whether MA was present or absent from the initial luminal perfusate; and 2. net secretory flux was linearly related to collection rate. Concerning the latter, it must be pointed out that collection rate is an approximation of perfusion rate, since the renal tubule might secrete or reabsorb fluid. No attempt was made to measure fluid movement. Clearance studies on whole fish indicate that MA secretion is inversely related to fluid reabsorption (Booz, unpublished observations). The present finding that the final perfusate to bath concentration ratio was linearly related to collection rate may reflect that fact.

Carbonic anhydrase has been implicated in proton secretion by renal tubules. The inhibitory effect of methazolamide on MA secretion suggests that pH gradients influence MA secretion. Note, however, that rapid perfusion -- as in the present study -- of a buffered solution through relatively short tubular segments would have precluded development of a transepithelial pH gradient.

This investigation was supported in part by a grant of the Whitehall Foundation to Arnost Kleinzeller.

XENOBIOTIC METABOLIZING SYSTEMS IN AQUATIC SPECIES; ACTIVITIES IN EXTRAHEPATIC TISSUES AND EFFECTS OF ELLIPTICINE ON HEPATIC MIXED-FUNCTION OXIDASES IN AQUATIC AND MAMMALIAN SPECIES

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Xenobiotic metabolism in aquatic species not only aids in the clearance of chemicals from tissues but can also produce toxic metabolites (carcinogens/mutagens) from ingested chemicals. Recently much work has been done on these systems in

liver of several aquatic species by a variety of laboratories including this one. However, studies on xenobiotic metabolizing systems in extrahepatic tissues have been few in number, and often limited to kidney and to freshwater species (see review by J.R. Bend and M.O. James in Biochemical and Biophysical Perspectives in Marine Biology, Vol. 4, ed. by D.C. Malins and J.R. Sargent, Academic Press, N.Y., 1978, p. 145). In mammalian species, extrahepatic xenobiotic metabolism has been extensively studied in the lung, skin, and intestine in our laboratory. In all cases, such metabolism seems to play an important role in chemical damage to those tissues. We have also been interested in the response of xenobiotic metabolizing systems in different tissues to exposures of the animal to chemicals and drugs which induce these systems in liver. In several cases, marked differences between liver and other tissues have been seen. In some mammals, lung, skin, and intestinal xenobiotic metabolism have responded to polycyclic hydrocarbons in ways similar to the liver. Our previous studies in aquatic species have indicated that hepatic systems may be responding to environmental pollutants (see Bend et al., Bull. MDIBL 19: 111, 1979). We have sought in this year's studies to compare certain marker enzymes for responsiveness in extrahepatic and hepatic tissues in flounder which have been treated with polycyclic hydrocarbons or in flounder caught in nature that seem to have hepatic enzymes affected by some source of pollution yet unidentified. Our results with intestinal enzymes are described in a separate report in this Bulletin by Foureman et al. The present report concerns our studies on some xenobiotic metabolisms in flounder and eel skin and on the response of the hepatic enzyme activities, 7-ethoxycoumarin de-ethylase (7EC) and NADPH-cytochrome c reductase to the cytochrome P-450 probe, ellipticine, in both aquatic and mammalian species.

METHODS

Most enzyme methods/assays have been described before (Bend et al., Bull. MDIBL 18: 60, 1978). A micro assay for 7EC and 7ERF (7-ethoxyresorufin de-ethylase) metabolism was used (J.R. Fouts in Fine Needle Aspiration Biopsy of the Rat Liver, ed. by G. Zbinden, Pergamon Press, Oxford, 1980, pp 33-37). NADPH-cytochrome c reductase was assayed as described in (Fouts and Devereux, J. Pharmacol. Exptl. Therap. 183: 458, 1972). Skin homogenates and microsomes from these were prepared as described in our publication on mammalian skin (Pohl et al., Drug Metab. Disp. 4: 442, 1976). Intestinal preparations are described in our paper (Chhabra et al., Drug Metab. Disp. 4: 208, 1976). Species used were the winter flounder (P. americanus), the little skate (R. erinacea), and freshwater eel (A. rostrata), all caught locally. Mammals were the Sprague Dawley rat and the hamster--tissues donated by Dr. Bodil Schmidt Nielsen. Ellipticine was donated by the National Cancer Institute and was dissolved in dimethylsulfoxide/water 1:1 for use in 7EC and reductase assays.

RESULTS AND DISCUSSION

Skin enzymes in flounder and eel: Skin homogenates prepared from both dark and light skin of flounder and from the entire skin of the eel were assayed for 7EC, 7ERF, aryl hydrocarbon hydroxylase (AHH), NADPH-cytochrome c reductase, and glutathione transferase (GSHTase) activity at 25°C. Microsomes were also prepared from these homogenates and assayed for 7EC, AHH, and reductase. Homogenates of skin of flounder with high hepatic 7ERF activity were compared with skin homogenates from flounder with low hepatic 7ERF activity. Comparisons were also made of skin from flounder treated (dosed i.p.) with the xenobiotic metabolizing enzyme inducers, 1,2,3,4-dibenzanthracene (DBA) or β-naphthoflavone (β-NF), and skin from untreated fish. Enzymes assayed in these skin preparations were 7EC and AHH. Liver from these β-NF- or DBA-treated fish had markedly elevated AHH, 7ERF and 7EC activities.

In no case, was any detectable 7EC, 7ERF, or AHH seen in any sample--either homogenate or microsomes, in either dark or light skin from flounder, in flounder with high or low hepatic 7ERF activity or in "induced" versus "control" flounder. Nor were skins from eel any different--no detectable enzyme activity was seen in any of the freshly caught eel, though their liver activity was quite normal. Total numbers of flounder tested were greater than 20. Both sexes were used and fish of widely different weights (low 180 g, high 500 g) were tested. Only 5 eels were studied, but here too the results (no MFO detectable) were the same. In some experiments, homogenates or microsomes from skin were added to liver in assays of hepatic MFO activity, and no inhibition (by the added skin) of these hepatic systems was seen--i.e., skin did not contain endogenous maskers of any MFO activity which might be present. Enzymes which were detectable in both flounder and eel skin homogenates were the GSHTase (with 2,4-dinitrochlorobenzene as substrate) at about 1/10 that in liver and, in microsomes, some very low NADPH-cytochrome c reductase activity--about 1/20 to 1/50 that seen in liver microsomes in both species. The NADPH-cytochrome c reductase activity of skin microsomes was not apparently different in dark versus light skin of flounder, and was not correlated at all with high versus low hepatic 7ERF activity either in untreated or treated (with DBA or βNF) flounder. These results are in marked contrast to mammals where low, but easily measured, MFO activity (e.g., 7EC, AHH) is seen in skin, and this is often correlated with hepatic MFO levels (high skin MFO in the same animals with high liver MFO) and is inducible in skin by polycyclic hydrocarbons like βNF (Pohl et al., Drug Metab. Disp. 4: 442, 1976).

Effects of ellipticine on 7EC metabolism in liver of several species: Ellipticine (5,11-dimethyl-[6H]-pyrido-[4,3b]carbazole) is a plant-derived antineoplastic that also is used to probe the cytochrome P-450-dependent MFO systems. It belongs to a class of chemicals giving type II difference spectra when added to microsomes, has a very high affinity (Ks < 1 μM) for the cytochrome P-450, and inhibits MFO activity by apparently blocking the transfer of electrons from NADPH to the P-450 via NADPH-cytochrome P-450 reductase. Transfer of electrons from the flavoprotein reductase to exogenous cytochrome c is not blocked by ellipticine. In mammals, ellipticine appears to have similar affinity for induced versus uninduced forms of P-450, whereas another probe, αNF (or 7,8-benzoflavone), is much more inhibitory to polycyclic hydrocarbon-induced P-450 forms than others, and has been used by Dr. Bend to detect induction of AHH in fish (see Bend et al., Bull. MDIBL 19: 111, 1979). Further details about ellipticine as a P-450 probe are found in (Guenthner et al., Biochem. Pharmacol. 29: 89, 1980).

We first verified that ellipticine was not inhibitory to fish liver NADPH-cytochrome c reductase at any concentration used (up to 10^{-5} M)--using homogenates of liver or hepatic microsomes from all species examined. Some results of tests of effects of ellipticine on 7EC metabolism in all 5 species studied are given in Figure 1. Essentially 2 kinds of response were seen--enzymes which were only minimally inhibited at 10^{-6} M versus those which were very sensitive to ellipticine at 10^{-6} M. The rat, eel and skate fell into a class of "resistant" species--whose hepatic metabolism of 7EC was inhibited markedly only by concentrations of ellipticine above 10^{-6} M, and even at 10^{-5} M was not completely shut down. The flounder and hamster enzymes were inhibited markedly by very low concentrations of ellipticine--even at 10^{-7} M there was about 50 percent inhibition and at 10^{-6} M there was very little enzyme activity measurable.

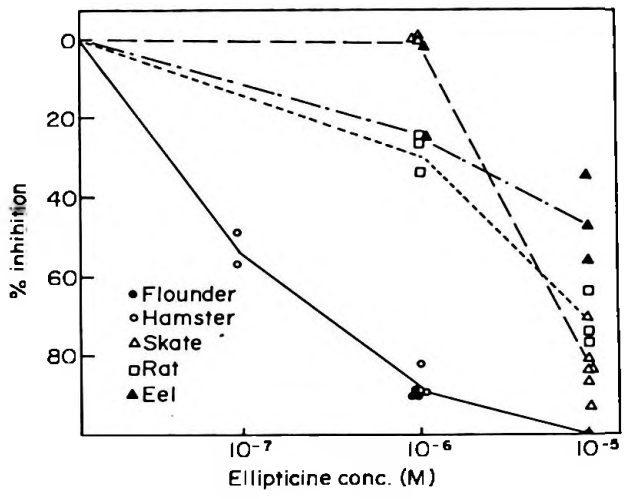

Figure 1

These results indicate that ellipticine may be a valuable probe of the differences in cytochrome P-450 between species and suggest specifically that the cytochrome P-450s of skate and flounder liver may be quite different.

ANOXIC TOLERANCE OF ATLANTIC HAGFISH (MYXINE GLUTINOSA) CARDIAC MUSCLE

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Atlantic hagfish have multiple hearts and a partially open circulatory system with sinuses reminiscent of those found in the invertebrate phyla. The two primary pumps, systemic (branchial) and portal-vein hearts, are composed of true vertebrate cardiac muscle which shows a myogenic origin of rhythmic heartbeat. Unlike most other non-cyclostome vertebrates, however, hagfish completely lack a coronary circulation for delivery of well-oxygenated blood to the cardiac musculature. Both hearts exclusively pump mixed venous blood which, even when fully-oxygenated, has an extremely low oxygen carrying capacity (1 ml O_2/100 ml blood). Functional characteristics of the respiratory pigments from hagfish further suggest chronic hypoxia of the cardiac tissue. Unlike higher vertebrates the hemoglobin of these animals is monomeric (rather than tetrameric) and, accordingly, does not display Bohr or Root effects to aid oxygen dissociation. The P_{50} values of both whole blood and hemoglobin solutions are extremely low, less than 5 mmHg PO_2 (Bauer et al., Nature 256: 66-68, 1975). All factors imply that cardiac muscle of hagfish continuously functions under in vivo conditions that are severely hypoxic by typical vertebrate standards.

The major objectives of this study were to determine: 1. the normal oxygen tensions available to working hagfish cardiac muscle; 2. the mechanical performance of hagfish systemic heart under normoxia and anoxia, and 3. a biochemical index (pyruvate kinase/cytochrome oxidase activity ratio; Gesser and Poupa, Comp. Biochem. Physiol. 48A: 97-103, 1974), which is highly correlated with anaerobic work capacity in hagfish heart compared with that of the obligately aerobic heart of Atlantic cod (Gadus morhua).

METHODS

Atlantic hagfish were captured with baited traps off St. Andrews, Canada, transported to M.D.I.B.L. and held in circulating fresh seawater tanks at ambient temperatures. Atlantic cod were captured with baited lines off Southwest Harbor, M.D.I. Cod hearts were immediately removed and held on ice until enzyme assays were performed that day.