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## HEPATIC CHEMICAL AND DRUG METABOLIZING ENZYMES IN COASTAL MAINE MARINE SPECIES

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This work is a continuation of our study of the hepatic and extrahepatic mixed-function oxidase system as a possible mechanism in marine species for dealing with increasing contamination of coastal waters by foreign organic chemicals from agriculture, industry, and urban waste.

Marine species collection, storage, and preparation of microsomal suspensions were carried out as previously described (Bend, Pohl, Fouts, Mt. Desert Is. Biol. Lab. Bulletin, 12, 12, 1972). Body and liver or hepato-pancreas weight ranges of the species used (see Table 1) were rock crab, 120-190 g, 2.8-6.7 g; lobster, 435-620 g, 18.4-29.3 g; eel, 110-180 g, 0.9-2.1 g; mummichog, ≈ 1 g, ≈ 200 mg; winter flounder, 205-280 g, 1.6-2.4 g; King of Norway, 850-1300 g, 12.4-27.0 g; dogfish shark, 1280-1600 g, 96-162 g; little skates, 650-1300 g, 14.1-32.5 g; large skates, 1400-2400 g, 34.3-140.5 g; thorny skates, 1400-1500 g, 40.1-67.4 g; hagfish, 220-250 g, 4.8-5.3 g; mackerel, 510-770 g, 7.9-10.2 g. King of Norway liver had a bright orange color which concentrated in the fatty layer during centrifugation at 10,000 × g leaving a normal appearing aqueous supernatant. Dogfish shark liver was extremely fatty (floated in sea water) and homogenates of shark liver yielded only 1-3 mg of microsomal protein per g tissue compared to the 4-18 mg/g yield of microsomal protein from the livers of other species studied. Protein content of the microsomal preparations was assayed by the method of Lowry (Lowry, Rosenbrough, Farr, Randall, J. Biol. Chem. 193, 265, 1951).

Aniline hydroxylase and d-benzphetamine demethylase activites were measured as previously described (Bend, Pohl, Fouts, Mt. Desert Is. Biol. Lab. Bulletin, 12, 12, 1972). Benzpyrene hydroxylase was measured by assay of fluorescent metabolites at the excitation and emission wavelength maxima of 3-OH benzpyrene (Wattenberg, Leong, and Strand, Cancer Res., 22, 1120, 1962); quinine sulfate, 30 μg/ml in 0.1 N $H_2SO_4$, was used to standardize fluorescence units for benzpyrene metabolism assays so that one unit equals the fluorescent intensity of 3 μg quinine sulfate/ml in 0.1 N $H_2SO_4$. Incubation mixtures contained 0.1 M HEPES buffer, pH 7.6, 5 mM glucose-6-phosphate, 5 mM $MgSO_4$, 2 units glucose-6-phosphate dehydrogenase, 1.0 mM NADP, 2.0 mg bovine serum albumin and 0.2-1.0 mg microsomal protein in 2.5 ml total volume. The cofactor solution was allowed to generate NADPH for 10 minutes before addition of microsomes and 0.6 mM benzpyrene in 0.1 ml acetone. Incubations in 25 ml Erlenmeyer flasks were carried out at 30°C for 15 minutes under air in a Dubnoff metabolic incubator whose shaking rate was at least 100 rpm. Substrate blanks were prepared for use with each determination by adding benzpyrene to a concomitantly incubated flask (containing all components of the incubation mixture except substrate) after having stopped metabolism by adding 1.0 ml cold acetone. The deethylation of 7-ethoxycoumarin was measured by recording the appearance of the fluorescent metabolite, umbelliferone (Ullrich and Weber, Z. Physiol. Chem., 353, 1171, 1972); assays were run at 30°C in the temperature-controlled cell compartment of a Turner Model 430 spectrofluorometer. The 2.5 ml incubation mixtures contained 0.1 M HEPES buffer, pH 7.8, 5 mM glucose-6-phosphate, 5 mM $MgSO_4$, 0.25 mM NADP, 2.0 units glucose-6-phosphate

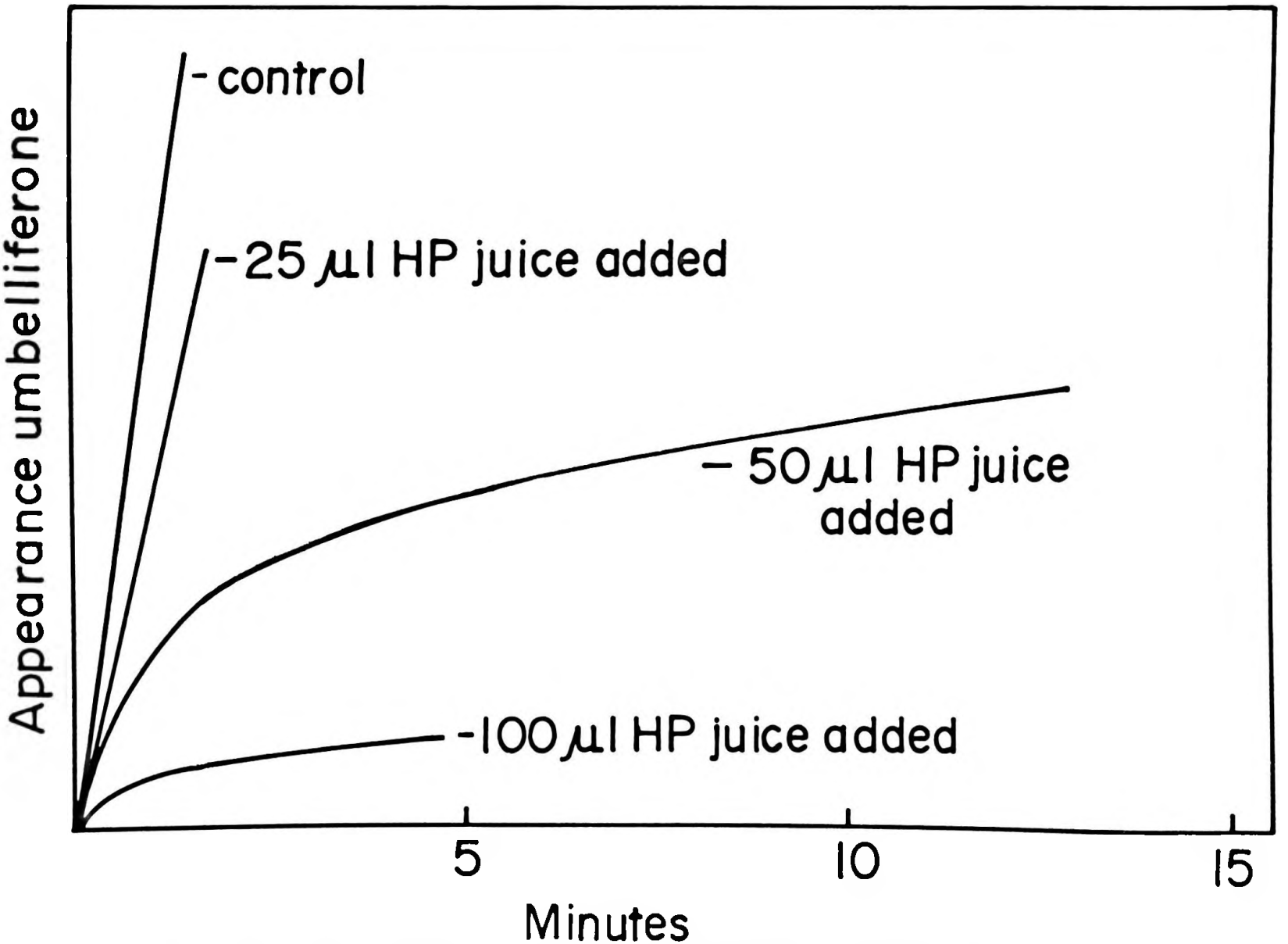




Figure 1: Inhibition of 7-ethoxycoumarin deethylating activity of skate microsomes by digestive juice from rock crab hepatopancreas.

TABLE 1

DRUG METABOLIZING ENZYME ACTIVITY IN MARINE SPECIES

| Species | Microsomal protein yield mg/gm liver or hepatopancreas | Activity (product formed/min/mg microsomal protein) Aniline hydroxylase[a] | d-Benzphetamine demethylase[a] | 7-Ethoxycoumarin deethylation | Benzpyrene hydroxylase[b] |
|---|---|---|---|---|---|
| Crustaceans | | | | | |
| Rock crab, *Cancer borealis* | 5 | 0.06,0.02 (2)[c] | <0.06[d] (2)[c] | <0.01[d] (2)[c] | <0.01[d] (2)[c] |
| Lobster, *Homerus americanus* | 3-8 | <0.01[d] (3) | <0.06 (3) | <0.01 (3) | <0.01 (3) |
| Cyclostomes | | | | | |
| Hagfish, *Myxine glutinosa* | 6-12 | 0.12 ± 0.05 (3) | 0.12 ± 0.03 (3) | 0.20 ± 0.07 (3) | 0.18 ± 0.02 (3) |
| Elasmobranchs | | | | | |
| Dogfish shark, *Squalus acanthias* | 1-3 | 0.07 ± 0.01 (3) | 0.15 ± 0.05 (3) | 0.08 ± 0.02 (3) | 0.07 ± 0.02 (3) |
| Little skate, *Raja erinacea* | 6-15 | 0.56 ± 0.19 (7) | 1.07 ± 0.19 (9) | 0.32 ± 0.14 (11) | 0.17 ± 0.10 (10) |
| Large skate, *Raja ocellata* | 6-8 | 0.49 ± 0.10 (3) | 1.49 ± 0.41 (3) | 0.47 ± 0.08 (3) | 0.30 ± 0.09 (3) |
| Thorny skate, *Raja radiata* | 5-6 | 0.16 ± 0.07 (3) | 0.45 ± 0.14 (3) | 0.12 ± 0.04 (3) | 0.12 ± 0.02 (3) |
| Teleosts | | | | | |
| Eel, *Anquilla rostrata* | 12 | 0.13 (1)[c] | 0.44 (1)[c] | 0.89 (1)[c] | 0.21 (1)[c] |
| Mummichog, *Fundulus heteroclitus* | 4-12 | 0.17,0.14 (2)[c] | 1.13 ± 0.76 (3)[c] | 0.49 ± 0.30 (3)[c] | 4.10 ± 2.10 (3)[c] |
| Winter flounder, *Pseudopleuronectes americanus* | 10-18 | 0.19 ± 0.05 (7) | 0.59 ± 0.13 (3) | 0.32 ± 0.25 (6) | 2.54 ± 1.67 (7) |
| King of Norway, *Hemitripterus americanus* | 10 | 0.01 ± 0.01 (3) | 0.16 ± 0.04 (3) | 0.06 ± 0.08 (3) | 0.004,0.02 (2) |
| Mackerel, *Scomber scombrus* | 4-8 | <0.01 (3) | <0.10 (3) | <0.01 (3) | <0.07 (3) |

[a] nmoles, mean ± S.D. (N)

[b] Fluorescence units, mean ± S.D. (N)

[c] Separate pools of liver or hepatopancreas from three or more individual animals.

[d] Minimum metabolism detected by the assay method used.

TABLE 2

INHIBITION OF DRUG METABOLIZING ENZYME ACTIVITY
OF SKATE MICROSOMES BY DIGESTIVE JUICE FROM HEPATOPANCREAS

| Hepatopancreas digestive juice | Added to 2.5 ml incubation mixture | Percent inhibition | | |
|---|---|---|---|---|
| | | Aniline hydroxylase | d-Benzphetamine demethylase | 7-Ethoxycoumarin deethylase[b] |
| Rock crab | 0 ul | 0 (0.99)[a] | 0 (1.40)[a] | 0 (0.62)[a] |
| | 5 | 39 | 21 | 0 |
| | 10 | 71 | 58 | 13 |
| | 25 | 92 | 75 | 37 |
| | 100 | 100 | 100 | – |
| Lobster | 0 | – | 0 (2.03)[a] | 0 (0.44)[a] |
| | 5 | – | 1 | 0 |
| | 10 | – | 6 | 5 |
| | 25 | – | 47 | 5 |
| | 100 | – | 100 | 18 |

[a]nmoles product formed/min/mg microsomal protein.

[b]Initial rates (see Figure 1).

dehydrogenase, 0.2 mM 7-ethoxycoumarin, and 0.2-1.0 mg microsomal protein (added last to start metabolism). Quench correction and calculation of deethylation rates were based on the fluorescence of umbelliferone standard solution added in 5 µl (to the complete incubation mixture including substrate) at the end of the assay period.

Table 1 summarizes our findings in a number of species arranged in evolutionary order. No simple correlation between habitat or eating habits and hepatic microsomal drug metabolizing activity results from this data. However the lack of detectable chemical metabolizing enzyme activity in mackerel liver suggested it would be profitable to investigate other wide-ranging marine species while the high benzpyrene hydroxylase activity in liver from winter flounder and mummichog may be indicative of pollution or polycyclic hydrocarbon induction of detoxicating enzymes in these inshore dwellers. We were unable to detect chemical or drug metabolizing enzyme activity in microsomal preparations from hepatopancreas of rock crab or lobster. The digestive juice that oozes from hepatopancreas during its gentle removal from the animal and during the short time it sits on ice awaiting homogenization proved to be a potent inhibitor when added *in vitro* to hepatic microsomal enzymes from livers of the little skate (Table 2). The shape of product appearance curves from the 7-ethoxycoumarin deethylation assay (Figure 1) suggested inhibition by hepatopancreas juice results from degradation of the deethylase enzyme. Addition of lobster or crab hepatopancreas microsomes

to little skate microsomes in incubation mixtures resulted in very little inhibition (data not shown) suggesting that the hepatopancreas inhibitor can be washed out of the hepatopancreas microsomes. Lobster green gland, the renal organ in crustaceans also contained no detectable microsomal 7-ethoxycoumarin deethylating activity. In mammals kidneys do possess some systems analogous to hepatic microsomal drug-metabolizing enzymes.

Devereux and Fouts have detected high levels of N-oxidation in mammalian liver and lung (Chemico-Biol. Interactions, in press). However no N,N-dimethylaniline (DMA) N-oxidation was found in liver microsomes from the following species: little skate, dogfish shark, King of Norway, mummichog, winter flounder, mackerel, hagfish, eel or lobster. DMA N-oxidase activity has been reported in liver homogenates from elasmobranchs of the Bimini area (Goldstein, Dewitt-Harley, Comp. Biochem. Physiol. 45, 895, 1973).

In summary this data revealed mixed-function oxidase activity in livers of all fish species studied except mackerel and suggested enzyme degradation by digestive juice as a possible reason for lack of mixed-function oxidase activity in hepatopancreas from crustaceans.

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## EPENDYMOMA OF THE CHOROID PLEXUS OF AN ELASMOBRANCH *Squalus acanthias*

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Neoplasms of elasmobranchs are quite rare and no tumors of the central nervous system have been reported in this class of animals. In mid-August of 1973 a two kg sexually mature male spiny dogfish *Squalus acanthias* was caught in Frenchman's Bay near Bar Harbor, Maine. A three mm in diameter tan, circumscribed tumor was projected dorsad from the mid-dorso-lateral surface of the right ala of the choroid plexus of the fourth ventricle. The choroid plexus is about nine mm wide, very thin, and transparent in this area over the medulla. The tumor was excised, fixed in formalin (10 percent in dogfish Ringer's solution), embedded in paraffin, sectioned at 6μ, and stained with hematoxylin and eosin. A small portion of the tumor was further fixed in 1.5 percent glutaraldehyde, postfixed in osmium tetroxide, embedded in Epon-Araldite, stained with lead citrate and uranyl acetate and examined in an electron microscope. Light microscopy revealed a differentiated well-vascularized papillary neoplasm growing from a hyperplastic region of choroid plexus. The tumor surface consisted of papillary projections covered by columnar cells (Figure 1). Crypts and acini lined by columnar cells were present throughout the tumor (Figure 2). Although numerous neoplastic cells filled some of the acinar lumina no invasion of the basement membrane was observed. The neoplastic cells contained abundant vacuolated cytoplasm, had indistinct borders and hyperchromatic pleomorphic nuclei. Mitotic figures were present in moderate numbers throughout the neoplastic mass. The ultrastructural studies revealed pleomorphic nuclei with marginated chromatin and cytoplasm that was quite vacuolated (Figure 3).

It is concluded that this is a benign neoplasm arising from the choroid plexus which can be best described by the general term ependymoma.

Over 1000 dogfish brains were dissected during the past five years including over 300 during