EFFECTS OF PHENOBARBITAL ON BILE SECRETION, GLUTATHIONE-S-ARYLTRANSFERASE ACTIVITY AND HEPATIC MIXED FUNCTION OXIDASE (MFO) PATHWAYS IN THE SMALL SKATE *Raja erinacea*.

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In mammals phenobarbital administration results in profound changes in hepatic function including enhancement of bile salt independent biliary secretion (Berthelot et al., Amer. J. Physiol. 219:809, 1970) and induction of MFO pathways (Conney, A.H. Pharmacol. Rev. 19:317, 1967). The present study was designed to determine if similar pharmacologic effects could be demonstrated in Elasmobranchs.

Small skates weighing approximately 1.0 kg were caught by net and randomized to a control group and treatment groups A and B. Control fish received daily intravenous injections of saline while treatment group A was injected with 7.5 mg phenobarbital. All fish were allowed to swim freely in large tanks for the period of study. After eight to ten days biliary cannulas were inserted and bile was collected for 48 hours as described (Boyer et al., Bull. MDIBL, 1973). The fish were then killed, the livers removed, placed on ice and weighed, and microsomes and cell supernatent prepared as described for determination of aniline hydroxylase and benzphetamine demethylase activity (Bend, Pohl and Fouts, Bull. MDIBL, 1972), benzpyrene hydroxylase and 7-ethyoxycoumarin deethylation activity (Pohl, Bend, Devereaux, and Fouts, Bull. MDIBL, 1973), and glutathione-S-aryltransferase activity (Biochem. J. 79:616, 1961).

TABLE 1

HEPATIC EFFECTS OF PHENOBARBITAL IN THE SMALL SKATE

	Non-operated Controls	Operated Controls	Phenobarbital Treated Skates (Group A)	(Group B)
Liver Weight/Body Weight	-------	.03 ± .009	.029 ± .009 (5)	.026 ± .007 (4)
Bile Flow (ml/kg/24 hr)	-------	3.12 ± .26 (6)	1.84 ± .85 (5)*	2.07 ± .57 (3)*
Aniline Hydroxylase[1]	.43 ± .09 (3)	.14 ± .08 (6)**	.06 ± .07 (4)	.25 ± .08 (3)
Benzphetamine[2] Demethylase	0.96 ± .14 (3)	.42 ± .19 (6)**	.28 ± .10 (4)	.71 ± .17 (3)
Benzpyrene[3] Hydroxylase	0.17 ± 0.1 (10)	.033 ± .023 (6)**	.012 ± .007 (4)	.13 ± .04 (3)***
7-Ethoxycoumarin[4] Deethylation	0.36 ± .15 (3)	.16 ± .12 (6)	.10 ± .07 (4)	.18 ± .06 (3)
GSH-S-Aryltransferase[5]	5.67 ± 4.84 (6)	7.24 ± 5.5 (6)	8.66 ± 6.7 (4)	6.04 ± 1.75 (4)

[1] nmoles p-aminophenol/min/mg microsomal protein
[2] nmoles/HCHO/min/mg microsomal protein
[3] units/min/mg microsomal protein
[4] nmoles umbelliferone/min/mg microsomal protein
[5] nmoles S-(2-chloro-4-nitrophenyl)glutathione formed/min/mg soluble protein

* Significantly less than controls ($p < .01$)
** Significantly less than non-operated controls ($p < .02$)
*** Significantly greater than operated but not non-operated controls ($p < .001$).

(No. of experiments are in parentheses)

As shown in Table 1 in contrast to results obtained in mammals, phenobarbital did not enhance the liver/body weight ratio and depressed rather than stimulated bile secretion. The latter was true whether the drug was administered daily (group A) or as a single dose eight days earlier (group B). No stimulating effects of phenobarbital on MFO pathways were observed in any of the studies. However, three of four MFO pathways were depressed in the operated control fish as compared with nonoperated controls suggesting that either the biliary cannulation or the length of fasting during captivity resulted in diminished microsomal enzyme function. Soluble enzyme activity as assessed by glutathione-S-aryltransferase was not affected in operated fish nor was it altered by phenobarbital administration. These studies indicate that phenobarbital does not stimulate biliary secretion or MFO pathways in the skate as it does in some mammalian species and suggests that the ability of this animal to increase hepatic metabolism and biliary excretion when chronically exposed to xenobiotics may be limited.

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SELECTIVE HEPATIC UPTAKE AND BILIARY SECRETION OF ORGANIC ANIONS IN ELASMOBRANCHS *Squalus acanthius* AND *Raja erinacea.*

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^{35}S-bromsulphathalein (BSP) or ^{14}C-Sodium Taurocholate (NaTC) were administered intravenously to free swimming dogfish sharks and to small skates to evaluate the capacity of these marine species to transport organic anions from plasma into bile. Plasma disappearance was determined by sampling blood at 15, 30, 60 and 120 minutes and at eight, 12 and 24 hours. Hepatic bile was obtained utilizing cannulation techniques which permitted intermittent sampling from the free swimming fish (Boyer, Bull. MDIBL 11:4-5, 1971). After 24 hours the fish were killed. Aliquots of plasma, bile, and liver homogenate were added to Scintisol and counted by liquid scintillation spectrometry. Samples of bile containing ^{35}S-BSP were also subjected to cellulose thin layer chromatography (n-butanol: glacial acetic acid: H_2O-40:10:50) to determine the percent of anions excreted as conjugates.

Both ^{35}S-BSP and ^{14}C-NaTC were removed from plasma by the liver and secreted into bile (Table 1). Initial fractional clearance rates of ^{35}S-BSP from plasma were .045 $\pm$ .008 in 6 dogfish and .044 $\pm$ .01 in four small skates while fractional clearance rates for ^{14}C-NaTC were .0319 $\pm$ .0015 and .050 $\pm$ .004 in dogfish and skates respectively. By 24 hours 79.1 percent and 78 percent of the administered doses of ^{35}S-BSP were recovered in the liver and bile of the dogfish and small skates respectively; these values compared favorably with recoveries of 80.5 and 59 percent of administered ^{14}C-NaTC in these two species. Large concentration gradients between plasma and bile were observed for both anions in dogfish and skates, averaging 4057:1 and 1592:1 respectively for ^{35}S-BSP, and 4180:1 and 2071:1 for ^{14}C-NaTC. When conjugates of ^{35}S-BSP were assessed by thin layer chromatography only 12.4 percent of the secreted BSP was metabolized by the dogfish while 24.7 percent of the excreted BSP was in conjugated form in bile from the small skate. Thus conjugation of BSP was not essential for transport into bile in either species.