barbital and polychlorinate biphenyls (Arclor) have been shown to be potent inducers of hepatic microsomal enzyme activity in rats (Chen and DuBois, Toxicol. Appl. Pharmacol. 1973, in press) the activities of these enzymes were also determined in fish pretreated with either sodium phenobarbital, Aroclor 1254 or Aroclor 1260, 50 mg/kg i.p for 5 days. As shown in Table 2 no appreciable inducing effect by either sodium phenobarbital or PCB was found. However all fish receiving phenobarbital showed definite signs of sedation.

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INVESTIGATIONS OF THE BIOSYNTHESIS OF L-ASPARAGINE IN THE SKATE *Raja ocellata* AND DOGFISH *Squalus acanthias*

D.A. Cooney, H.A. Milman and A.M. Guarino, Laboratory of Toxicology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

In a previous communication we reported the apparent absence of L-glutamine-dependent L-asparagine synthetase in the organs of several fish (MDIBL Bull. 12, 68, 1972). At the same time extracellular L-asparagine in at least one of the species examined was abundant. To investigate the source of this L-asparagine we have introduced six analytical changes designed to enhance the likelihood of detecting L-asparagine synthetase in fish: 1) more concentrated homogenates were prepared; 2) the homogenates were dialyzed against 30 percent buffered glycerin to further concentrate the enzymes in them and to remove putative low-weight inhibitors; 3) whole homogenates and not simply supernatants were used for the analyses in the event that the synthetase was particulate (Biochem. Pharm. 21, 39, 1972); 4) in addition to L-glutamine ammonia was examined as precursor of the future amide of L-asparagine (J. Biol. Chem. 247, 6708, 1972); 5) the cyanogenic route to L-asparagine, which operates in many plants (J. Biol. Chem. 244, 2632, 1969) and in several species of bacteria (J. Am. Chem. Soc. 85, 2874, 1963), also was studied in these homogenates:

$$\text{L-cysteine} + \text{HCN} \longrightarrow \beta\text{-cyano-L-alanine}$$

$$\beta\text{-cyano-L-alanine} + H_2O \longrightarrow \text{L-asparagine;}$$

6) ammonium chloride was added to a final concentration of 0.1M in select cases with the intention of inhibiting the L-asparaginase known to be present in the body fluids of the fish used and of thereby preventing the artifactual hydrolysis of the product of the enzyme under study (Biochim. Biophys. Acta 185, 228, 1969).

Fish were killed by decapitation or exsanguination and their organs frozen at -20° until the time of analysis. L-asparagine synthetase was measured radiometrically as described previously (MDIBL Bull. 12, 68, 1972). In the present study for increased sensitivity L-asparaginase also was measured radiometrically using [U-^{14}C]-L-asparagine as substrate. The [U-^{14}C]-L-aspartic acid arising from the hydrolysis of the amide was β-decarboxylated essentially as described previously (J. Lab. Clin. Med. 81, 455, 1973). This technique is capable of detecting as little as 1×10^{-5} I.U./ml of L-asparaginase in a 5 μl sample. The lithium citrate system of Oreskes was used to separate

β-cyano-L-alanine (Calbiochem, California, U.S.A.) from L-aspartic acid on the JEOLCO Amino Acid Analyzer (Anal. Chem. 37, 1720 1965). β-cyano-L-alanine synthetase was measured by a new radiometric technique patterned after the L-asparagine synthetase assay. Briefly Na ^{14}CN, 1 μCi, L-cysteine, 50 nmoles, Tris HCl, pH 7.4, 100 nmoles in a volume of 10 μl were added to 5 μl of 1:2 (w:v) homogenate of the fish organ (prepared in the medium described by Meister (J. Biol. Chem. 247, 6708, 1972) and dialyzed at 4° for 12 hours against a large excess of the same medium rendered 30 %, v:v in glycerin). The incubations were allowed to proceed for 30 minutes at 37° whereafter the reaction vessels were opened and heated at 95° for 25 minutes in the hood to dissipate unreacted cyanide. After cooling 50 μl of 0.66M sodium acetate, pH 5.0 was added and the vessels left at room temperature in the hood overnight. Then one I.U. of glutamate oxaloacetate transaminase, one μmole of α-ketoglutarate, one μmole of zinc sulfate and 10 I.U. of L-asparaginase from *E. coli* were added to the reaction mixture and 5 μl of a saturated solution of $Ba(OH)_2$ added to the underside of the lids of the vessels which then were closed. After three hours at 37° the lids were removed and $^{14}CO_2$ entrapped as $Ba^{14}CO_3$ quantitated by scintillation spectrometry.

For the inhibition of L-asparaginase ammonium chloride was added to representative samples to a final concentration of 0.1M. This additive did not interfere with subsequent radiometric analysis. L-asparagine pools were measured in neutralized five percent perchlorate extracts. Digestion of the protein pellets from these extracts was accomplished with 0.1 percent Pronase at 45° for four hours. L-aspartic acid and L-asparagine liberated in this step were analyzed spectrophotometrically.

No evidence was found for the presence of L-glutamine-dependent or ammonia-dependent 'mammalian-type' L-asparagine synthetase in the several organs of these two species of fish. β-cyano-L-alanine synthesis also was absent. L-asparaginase could be detected radiometrically in the organs of both species although at higher concentration in the skate. Attempts to inhibit this hydrolase with ammonium ions via product inhibition also failed to uncover any L-asparagine synthesis. Nevertheless L-asparagine was identified by automatic amino acid analysis in the tissue fluids and proteins of both species. The route used to synthesize this amino acid in fish does not appear to be the same as that operative in mammals, plants or bacteria.

It is possible that marine species amidate L-aspartic acid on its β-carboxyl only after esterification to tRNA or that neither L-aspartic acid nor L-cysteine serve as the source of the carbon-skeleton of L-asparagine in fish. Even if L-aspartic acid is the true precursor some amide-donor other than ammonia or L-glutamine may be used. Lastly it is conceivable that these elasmobranchs do not in fact synthesize L-asparagine at all but absorb it from the gut or through the gills. In this case, L-asparagine would be an essential amino acid and the skate and dogfish would be expected to be extremely prone to the toxic actions of L-asparagine-depletion. The finding that parenteral L-asparaginase at a dose of 10,000 I.U./kg produced no overt toxicity in the dogfish only seems to accentuate the problem.