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30. Supported by a grant from the Cornell Biotechnology Program, which is supported by the New York State Science and Technology Foundation and a consortium of industries. N.F. is on leave of absence from Osaka University School of Medicine. We thank K. Verner and D. Gross for the microphotography, B.-K. Tye for the yeast strain, G. Schatz for the antibody against porin, and B. Hall for the yeast expression vector pMAC561. The entire structural gene for the α subunit of the *T. californica* acetylcholine receptor was isolated as a cloned cDNA fragment by T. Claudio.

11 July 1985; accepted 30 October 1985

Monooxygenase Induction and Chlorobiphenyls in the Deep-Sea Fish *Coryphaenoides armatus*

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Inhibition of liver microsomal ethoxyresorufin *O*-deethylase and aryl hydrocarbon hydroxylase activities by α-naphthoflavone and by polyclonal antibodies to hydrocarbon-inducible cytochrome P-450E from teleost liver indicated a xenobiotic-induced origin of these activities in the deep-sea fish *Coryphaenoides armatus*. Specific recognition of a protein by antibodies to P-450E in an immunoblot assay further indicated xenobiotic-induced cytochrome P-450 in these animals. Levels of apparently induced cytochrome P-450 and monooxygenase activity correlated positively with the tissue content of chlorobiphenyls of known inducing activity, implicating such compounds in biochemical effects occurring in the deep ocean.

REGIONS OF THE DEEP SEA BELOW 1000 m are generally more stable environments than shallower continental shelf and coastal regions (*1*). Recent evidence, however, shows the potential for coupling between surface ocean biological activity and deep ocean organisms (*2*). Previous studies revealed a penetration of the deep sea by foreign compounds originating from land-based activities (*3*). Deep-sea waste disposal now under consideration (*4*) would contribute further to the occurrence of such compounds.

Cytochrome P-450 monooxygenases catalyze transformation of many foreign compounds, leading to detoxification or bioactivation. Cytochrome P-450 isozymes having specific catalytic functions are induced in fish by 3-methylcholanthrene (MC), β-naphthoflavone (BNF), other hydrocarbons, and certain halobiphenyls (*5*). These induced proteins and their activities detected in feral fish in coastal waters have been attributed to the action of such chemicals (*6*). Our analyses of cytochrome P-450 systems indicate that some deep-sea fish have an induced capacity to biotransform xenobiotics and, therefore, that biochemical effects of xenobiotics are now occurring in the deep ocean.

A widely distributed deep-sea fish, the rattail *Coryphaenoides armatus*, was sampled from two sites in the western North Atlantic: near Hudson Canyon on the continental slope off New York and near Carson Canyon off Newfoundland (*7*). The animals were alive on retrieval, were of a similar size (40 to 80 g) at each site, and their gonads were not obviously developed. Genetic distinctions among fish from these sites are not likely to be large (*8*).

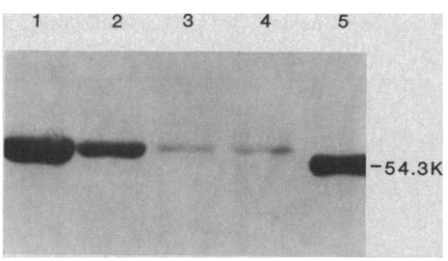

Fig. 1. Immunoblot of *C. armatus* liver microsomes with polyclonal antibody to P-450E. Microsomal proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose (*23*). Nitrocellulose was exposed to antibody to P-450E (1:500) followed by goat antibody to rabbit IgG and rabbit peroxidase-antiperoxidase (Bio-Rad peroxidase staining method). The same amount of microsomal P-450 (20 pmol) was added to lanes 1 to 4. Lane 1, Hudson Canyon fish (EROD, >2.0 pmol/min-pmol P-450); lane 2, Hudson Canyon fish (EROD, 0.8); lanes 3 and 4, Carson Canyon fish (EROD, <0.2 in each). Lane 5 contained 6.5 pmol of purified scup P-450E. Quantitation of cross-reacting material in *C. armatus* was not possible due to lack of standards, but intensity of staining with purified P-450E is linear to about 6 pmol.

Previous studies (*9*) showed that hepatic microsomal preparations of *C. armatus* had active electron transport components and native cytochrome P-450. Hepatic microsomes prepared and analyzed by established methods (*9*) had similar levels of microsomal cytochrome P-450 (about 0.30 nmol/mg) and of reduced nicotinamide adenine dinucleotide phosphate–cytochrome *c* (P-450) reductase activity (90 nmol/min-mg) in fish from both sites.

Ethoxyresorufin *O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) activities, which are catalyzed principally by MC- or BNF-inducible cytochrome P-450 isozymes in fish (*10*), were detectable in all samples but were significantly higher in animals near Hudson Canyon (Table 1). Levels of activity per nanomole of cytochrome P-450 (a measure of catalytic efficiency) in fish taken near Hudson Canyon were comparable in some individuals to the levels in MC-induced fish species from shallow waters, that is, greater than 2 nmol per minute per nanomole of cytochrome P-450 for EROD activity. AHH activity of Hudson Canyon samples was inhibited more strongly by α-naphthoflavone (ANF), a compound that preferentially inhibits catalytic activity of MC-inducible cytochrome P-450 in fish (*6, 10*) and mammals (*11*). By contrast, ethoxycoumarin *O*-deethylase (ECOD) activity differed to a lesser extent between these groups. In other fish, ECOD activity is catalyzed as much as 50 percent by other, noninducible isozymes (*12*).

The conclusion that the EROD and AHH activities in *C. armatus* reflect the presence of induced cytochrome P-450 was strongly supported by analysis with polyclonal antibodies to the MC-inducible liver cytochrome P-450 (cytochrome P-450E) (*12*) from the teleost *Stenotomus chrysops*. These antibodies inhibit EROD activity, and cross-reactivity is evident in enzyme inhibition and immunoblotting with similarly induced isozymes in other fish species (*12, 13*). Antibodies to P-450E preincubated with *C. armatus* liver microsomes inhibited EROD activity progressively in titration, up to 45 ± 3 percent ($n = 3$) at a ratio of 60 μg of purified immune immunoglobulin G (IgG) per picomole of P-450, compared to activity with preimmune IgG (*14*). Immunoblotting of *C. armatus* microsomes revealed that antibodies to P-450E recognized a protein with a molecular weight of about 55,000 (55K), in the range (54K to 58K) of

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induced cytochrome P-450 in other fish. This protein was present in greater amounts in fish with higher EROD activity, such as those from Hudson Canyon (Fig. 1). On the basis of these features and the specificity of antibodies to P-450E in immunoblotting (*13*), we conclude that this protein is an MC-induced type cytochrome P-450.

The distinction between *C. armatus* from the two sites indicates a greater response to MC-type inducers near Hudson Canyon. Gas chromatography–mass spectrometry (GC-MS) (*15*) revealed that the liver of *C. armatus* from near Hudson Canyon contained polychlorinated biphenyls at concentrations (Table 2) similar to the tissue content known to strongly induce AHH activity in other fish (*16*). The specific chlorobiphenyls identified thus far (Table 2) include appreciable amounts of 2,3,3′,4,4′-pentachlorobiphenyl and 2,3,3′,4,4′,5-hexachlorobiphenyl, both of which have MC-type inducing capacity in mammals, although as mixed-type inducers (*17*). Residue levels in fish from Carson Canyon were significantly less for several chlorobiphenyls, including the two just mentioned. Other unidentified compounds could be influencing monooxygenase systems in the deep sea, yet the present results establish a plausible chemical basis for the apparent induction.

Several of the chlorobiphenyls (Table 2), as well as *pp′*-DDE and *pp′*-DDD identified in these samples (*15*), are phenobarbital-type inducers. Fish generally show little or no induction by such compounds (*18*). Polychlorinated camphenes of a complexity consistent with the pesticide Toxaphene, whose effects on cytochrome P-450 in fish are unknown, were also detected. The biotransformation of specific chlorobiphenyls or other factors contributing to the patterns and tissue content of residues have yet to be assessed. The difference between Carson Canyon and Hudson Canyon fish could reflect the proximity of the latter to urban centers or known dump sites up-canyon on the adjacent continental shelf (*4*).

This apparent induction of cytochrome P-450 provides evidence for effects of widely distributed organic xenobiotics in the deep ocean. The significance of this induction, whether adaptive or maladaptive, is not known, but the potential exists for further biological change.

The data also describe hepatic enzymes in deep-sea fish that appear to be as catalytically efficient as those in shallow water species. This contrasts with other enzymes that have lower catalytic efficiency in the deep sea, such as the well-studied enzyme muscle lactate dehydrogenase (*19*), and could reflect the different biological functions and regulation of these enzymes. Interactions between substrate and catalyst in xenobiotic monooxygenase systems are characterized by hydrophobic associations (*20*), which in other systems are known to be sensitive to changes in pressure (*21*). Pressure could also influence membrane function (*21*), affecting requisite protein-protein interactions between cytochrome P-450 and cytochrome P-450 reductase in the membrane (*20*). Microsomal monooxygenase systems offer distinct possibilities for the study of structural and functional adaptations of membrane-bound proteins in the deep sea.

Specific polyclonal antibodies to cytochrome P-450E and the monoclonal antibodies to cytochrome P-450E (*22*) could be applicable in studies of such adaptation. As demonstrated here, such antibodies are useful in analyzing cytochrome P-450 induction as a measure of xenobiotic effects in fish of various species and from various environments.

Table 1. Monooxygenase activities in *C. armatus* liver microsomes. Analyses (*10*) were accomplished under reaction and temperature (20°C) conditions determined to be optimal for *C. armatus* monooxygenase activity. Some Hudson Canyon samples analyzed before (*9*) were reanalyzed here, with identical results. A fifth sample from Hudson Canyon had an EROD activity of 0.75 nmol/min per nanomole of P-450, consistent with other values in that group. That sample also had a large amount of cytochrome P-420 and was not included in the calculations. Values are means ± standard errors.

Activity (nmol/min-nmol P-450)	*C. armatus* source	
	Hudson Canyon ($n = 4$)	Carson Canyon ($n = 8$)
EROD	1.175 ± 0.310*	0.178 ± 0.050
AHH	0.408 ± 0.170*	0.045 ± 0.010
ECOD	0.164 ± 0.080	0.097 ± 0.010
AHH inhibition by $10^{-4}M$ ANF (% activity remaining)	4 ± 1†	38 ± 9

*Significantly different from value for Carson Canyon ($P < 0.01$, two-sided Mann-Whitney U test). †$P < 0.02$.

Table 2. Chlorobiphenyl concentrations in *C. armatus* liver from fish in the Hudson Canyon and Carson Canyon areas. Values (grams × 10^{-9} per gram of wet weight) are means ± standard errors. Detection limits were in the range 0.01 × 10^{-9} to 0.06 × 10^{-9} g/g depending on the sample size. IUPAC, International Union of Pure and Applied Chemists.

Chlorobiphenyl IUPAC number (*24*)	Substitution pattern	Hudson Canyon ($n = 5$)	Carson Canyon ($n = 2$)
28	2,4,4′	6 ± 3	3.3 ± 0.1
44	2,2′,3,5′	23 ± 20	0.6 ± 0.6
49	2,2′,4,5′	27 ± 9	10 ± 7
52	2,2′,5,5′	47 ± 15	8.5 ± 3.2
60	2,3,4,4′	9 ± 2	3.4 ± 2.2
70	2,3′,4′,5	18 ± 4	5.1 ± 2.4
86	2,2′,3,4,5	27 ± 14	3.2 ± 3.2
87	2,2′,3,4,5′	16 ± 5	6.7 ± 2.6
95	2,2′,3,5′,6	123 ± 41*	8.8 ± 0.7
101	2,2′,4,5,5′	200 ± 63	52 ± 31
105	2,3,3′,4,4′	129 ± 26*	9.9 ± 0.6
128	2,2′,3,3′,4,4′	44 ± 27	3.8 ± 2.7
129	2,2′,3,3′,4,5	68 ± 29*	6.0 ± 3.4
137	2,2′,3,4,4′,5	46 ± 11*	5.6 ± 0.8
138	2,2′,3,4,4′,5′	596 ± 118*	69 ± 37
141	2,2′,3,4,5,5′	15 ± 5	3.8 ± 1.6
153	2,2′,4,4′,5,5′	570 ± 120*	77 ± 40
156	2,3,3′,4,4′,5	54 ± 15*	7.1 ± 1.6
180	2,2′,3,4,4′,5,5′	352 ± 64*	46 ± 13
183	2,2′,3,4,4′,5′,6	120 ± 41*	11 ± 5
194	2,2′,3,3′,4,4′,5,5′	59 ± 11*	5.4 ± 3.5
195	2,2′,3,3′,4,4′,5,6	60 ± 23*	8.1 ± 2.1
206	2,2′,3,3′,4,4′,5,5′,6	53 ± 8*	4.8 ± 2.8
207	2,2′,3,3′,4,4′,5,6,6′	10 ± 2*	1.0 ± 0.6
Total		2730 ± 550*	360 ± 144

*$P < 0.05$, Mann-Whitney U test.

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13. Precipitin formation on double-diffusion analysis was evident only with cytochrome P-450E and not other isozymes from scup. Antibodies to P-450E reacted only with P-450E on immunoblot of the five forms of P-450 representing the total complement resolved from scup liver. A single band in the P-450 region coincident with P-450E (54.3K) was recognized in BNF-induced scup. Cross-reactivity of antibodies to P-450E was indicated by strong reaction with a single band in liver microsomes of BNF-treated trout (*Salvelinus fontinalis*). The same amount of microsomal P-450 from control trout had barely detectable staining.
14. The lesser inhibition of EROD activity suggests a lower affinity of antibodies to P-450E for the enzyme in *C. armatus*.
15. Chlorobiphenyls and chlorinated camphenes were identified by glass capillary GC and glass capillary GC-MS (EI and pulsed positive-negative ion chemical ionization with CH_4). Chlorobiphenyls were quantified by glass capillary GC by using response curves generated by a standard of each chlorobiphenyl. An SE-52 column (J & W Scientific) installed in a Carlo Erba Model 2150 gas chromatograph equipped with a split-splitless injector and ^{63}Ni electron-capture detector interfaced with a Columbia Scientific Instruments Supergrator 3 electronic integrator was used. A Finnegan 4510 quadrupole mass spectrometer interfaced with a Carlo Erba 4160 gas chromatograph and a Finnegan INCOS 2300 data system and standard EI/CI ion source and PPNICI accessory was used for GC-MS analyses. Further details are available from the authors. Abbreviations: *pp′*-DDE: 1,1′-(2,2-dichloroethenylidene)bis[4-chlorobenzene]; *pp′*-DDD: 1,1′-(2,2-dicloroethylidene)bis[4-chlorobenzene].
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19 August 1985; accepted 20 December 1985

Characterization of Highly Immunogenic p66/p51 as the Reverse Transcriptase of HTLV-III/LAV

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Approximately 80 percent of all human sera that react with antigens of HTLV-III, the etiologic agent of the acquired immune deficiency syndrome (AIDS), recognize protein bands at 66 and 51 kilodaltons. A mouse hybridoma was produced that was specific to these proteins. Repeated cloning of the hybridoma did not separate the two reactivities. The p66/p51 was purified from HTLV-III lysates by immunoaffinity chromatography and subjected to NH_2-terminal Edman degradation. Single amino acid residues were obtained in 17 successive degradation cycles. The sequence determined was a perfect translation of the nucleotide sequence of a portion of the HTLV-III *pol* gene. The purified p66/51 had reverse transcriptase activity and the monoclonal immunoglobulin G specifically removed the enzyme activity from crude viral extract as well as purified enzyme.

HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV-III/LAV) is the etiologic agent of the acquired immune deficiency syndrome (AIDS) (*1*). By the use of the Western blot technique, several antigens in lysates of purified HTLV-III have been recognized as major targets of antibody reactivity with sera of HTLV-III infected individuals (*2*). Antigens of 120, 66, 51, 41, 31, 24, and 17 kD are recognized in these blots (Fig. 1A). Of these antigens, those of 17 and 24 kD (p17 and p24) have been identified as proteins encoded by the HTLV-III *gag* gene, and gp120 and gp41 as products of the *env* gene (*3, 4*).

The nature of p51 and p66 remains to be determined. Reactivity to these proteins is present in at least 80 percent of randomly tested human sera that are HTLV-III antibody positive. These proteins are antigenically unrelated to HTLV-III *gag* proteins p24 and p17, or *env* proteins gp120 and gp41 (*5*). If one assumes that these proteins are encoded by the virus, they may be derived from a long open reading frame (LOR) other than the LOR's of the *gag* and *env* genes.

In one approach to this question we prepared hybridomas secreting monoclonal antibodies that recognized these two proteins. BALB/c mice were immunized by several intraperitoneal injections of detergent-lysates of purified HTLV-III strain 3B produced in H9 cells (*6*). Splenic lymphocytes were fused with the NS-1 mouse myeloma line as described (*7*). Supernatant fluids from the hybrids obtained were screened for antibody to HTLV-III proteins by an enzyme-linked immunosorbent assay (ELISA) in which we used detergent-disrupted HTLV-III as antigen. Hybrids that were scored positive in this assay were expanded and tested further by the immunoblot technique to define specificities.

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