Induction of Aryl Hydrocarbon (Benzo[a]pyrene) Hydroxylase in Fish by Petroleum

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A number of lipophilic substances including drugs, insecticides, carcinogens and steroid hormones induce liver microsomal mixed function oxidases in animals (GELBOIN 1967; CONNEY 1967). CLARKE and DIAMOND (1971) demonstrated the metabolism of benzo[a]pyrene in fish tissue and LEE et al. (1972a, 1972b) reported metabolism of benzo[a]pyrene and naphthalene *in vivo* by marine fish but not mussels. The existence of inducible aryl hydrocarbon hydroxylases (AHH) in fish may provide a convenient means of assessing previous exposure to petroleum or other products containing polycyclic aromatic hydrocarbons.

MATERIALS AND METHODS

Brown trout (*Salmo trutta*), 2-4 years old, were collected from a small remote lake on the Avalon Peninsula of Newfoundland that appeared to be free of any sources of contamination, and from a lake in the city of St. John's which is considered to be polluted by oil and other contaminants. Local residents have reported oil slicks entering this lake and sources of oil contamination have been identified. Capelin (*Mallotus villosus*), 2-4 years old, were collected at the seashore during the June 1974 spawning.

In all AHH measurements, liver and gills were taken from freshly-killed fish. Liver (0.5-2 g) was homogenized by hand in a 7 ml all-glass tissue grinder with 4 ml buffer (0.05 M Tris chloride-0.25 M sucrose, pH 7.5). Gills were ground in a mortar and pestle with 4 ml buffer and fine acid washed sand, followed by hand homogenization with sand in a 15 ml grinder. Homogenates were centrifuged for 10 min at 9000 x g and the supernatants frozen at -20°C and assayed within a week. The time required for this preparation was such that two days were required to complete some groups, but control and experimental fish were always taken in pairs. Aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity was assayed by the method of NEBERT and GELBOIN (1968); dilutions were made as necessary to bring the activity within the linear range of the assay. Protein was determined by the method of LOWRY et al. (1951) using bovine serum albumin as a standard. AHH activity units are arbitrary units of alkali-extractable

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fluorescence, measured with a Turner 110 fluorimeter using excitation filter no. 3 (360 nm maximum) and emission filter 7-60 (> 460 nm).

To demonstrate the induction of AHH activity, both brown trout (from the clean lake) and capelin were used; these fish were taken in May and June 1974 respectively. Basal levels of AHH specific activity were measured in livers of a small number of randomly-selected fish (Table 1). Groups of fish were kept in 300 liter recirculating tanks under dim light and an emulsion of oil was administered daily: One ml of Tia Juana Medium Venezuela crude oil was homogenized 10 min with 100 ml water. After settling 3 min, the bottom 85 ml was drawn off and added to the tanks. Oil concentrations measured fluorimetrically (KEIZER and GORDON 1973) throughout the experiment showed 1.4-1.6 mg/l 30 min after administration and 0.3-0.4 mg/l after 24 hours, i.e., an average exposure of about 1 ppm. We observed that the oil was continually removed by absorption to the sides of the tank and to the sand-charcoal filters.

Measurements of AHH levels found in the field were made on brown trout from the clean and polluted lakes. All fish were taken during the same two-week period in August 1974. Shortly afterwards, sediment samples were taken from the lakes, since sediment appears to act as an "integrator" of oil contamination levels (HARGRAVE, personal communication) which may fluctuate rapidly and widely in the water column itself. Samples of sediment (5 g wet wt) were extracted twice with 20 ml of methanol. After evaporation, the residue was dissolved in 1 ml chloroform, diluted into hexane, and the total fluorescence measured using the condition described above. The residue of sediment was dried for determination of dry weight. Thin layer chromatograms of the extracts were run on silica gel G plates (Macherey-Nagel) using benzene as the mobile phase.

RESULTS

In both trout and capelin, significant increases in the specific activity of liver AHH were observed within 17 days (Table 1). Activity in gills from treated fish differed significantly from control levels of virtually zero. There was no noticeable difference in mortality or behaviour between treated and control fish. (In preliminary experiments, no AHH activity could be demonstrated in muscle, blood, or skin; AHH activity was found in kidney and/or adrenal but was not monitored in these experiments due to the difficulty of distinguishing these organs.)

Specific activities of liver AHH of trout from the clean and "polluted" lakes were significantly different, indicating that environmental factors are reflected in the enzyme levels (Table 2). Whether these factors consist of, or include, oil contamination is difficult to prove in field measurements (see Discussion). The

TABLE 1

Exposure (days)	No. fish/group*	AHH specific activity (U/mg protein ± S.D.)			
		Liver		Gill	
		Control	Oil treated	Control	Oil treated
Trout (May 1974)					
0	2	102			
16-17	6	68 ± 14	240 ± 88 (t = 4.72, P < .001)	.07 ± .51	8.7 ± 6.1 (t = 3.46, P < .01)
Capelin (July 1974)					
0	6**	28.86			
7-8	4	27.2 ± 16.7	58.1 ± 17.3 (t = 2.57, P < .05)	.043 ± .083	1.07 ± 1.26 (t = 1.62, N.S.)
15-16	6	27.0 ± 10.7	130.6 ± 34.0 (t = 7.11, P << .001)	.037 ± .085	3.24 ± 1.05 (t = 7.45, P << .001)

*Sexes evenly distributed among groups.
**Livers pooled before homogenizing.

fish from the "polluted" lake had a strong and distinctly oily taste. The fluorescence extractable from the sediments was very much greater in the "polluted" than in the control lake (Table 2), and the pattern of blue fluorescence was distributed unevenly over the length of the thin-layer chromatograms, similar to chromatograms of whole crude oil. The clean pond sediments yielded a weak blue, narrow band near the solvent front. All sediment extracts showed a band of orange fluorescence at the origin which was not present in crude oil. Because of the filter arrangement used, this should have contributed to the fluorescence measured in all sediments.

TABLE 2

	AHH in trout liver (units/mg protein)	Extractable fluorescence in sediments (units/g)
Control pond (August 1974)	26.5 ± 19.4 (n = 8)	1053, 943 (n = 2)
Oil polluted pond (August 1974)	362 ± 51 (n = 3) (t = 16.8, P << .001)	11500, 12800 (n = 2)

DISCUSSION

We feel that after further development, aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity will serve as a useful index of recent or long-term oil exposure in fish. The time and circumstances required for the decay of this induced activity have not yet been determined (although fish from the polluted lake have maintained a high level of AHH activity during a year in captivity), and information on the specificity of induction remains to be gathered. It is unlikely that AHH will be found to be specifically induced by oil; other contaminants (e.g., polychlorinated biphenyls, aromatic pesticides) may be found to induce it. We feel, however, that AHH measurements will prove useful for the following reasons:

(1) Oil spills frequently occur in otherwise unpolluted locations, particularly marine oil spills, where interfering sources of pollution can be ruled out. The determination of AHH in fish at various distances from a pollution source will provide badly needed information on the range of influence of the biological effects of an oil spill.

(2) There is no consensus on a definition of chronic or low-level oil contamination determinable by any procedure. No single constituent, or even group of constituents, is unequivocally associated with, and only with, oil pollution. AHH measurements may take a place in the repertory of procedures currently in use and under development for the assessment of oil contamination; compared with the direct measurements of petroleum constituents in fish tissue, AHH measurement has the advantages of simplicity and adaptability to automation.

(3) To date, there has been little success in proving direct physiological effects of petroleum contamination on fish. The induction of mixed function oxidases by chlorinated aromatic pesticides has been implicated in disturbances of reproductive physiology in birds, apparently by way of altered hepatic steroid metabolism (PEAKALL 1967, 1970). Similarly the effect of induction on the activation (MILLER 1970; GELBOIN et al. 1972) or detoxification (GELBOIN 1967; CONNEY 1967) of carcinogenic polycyclic aromatic hydrocarbons in petroleum remains to be determined.

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