

centration. This has been reported to be the case in several mammalian cytochrome P-450 preparations (*Chem-Biol. Interactions* 9:169, 1974; *Pharmacologist* 17:185, 1975). Evidence for incomplete reduction of cytochrome P-450 in preparations devoid of cytochrome b₅ is a peak of absorption at 423 nm in the absolute spectrum of dithionite-reduced preparations complexed with carbon monoxide. A significant peak at 423 nm was observed in the majority of the spectra obtained for skate liver cytochrome P-450.

We have been able to solubilize and partially purify cytochrome b₅ from skate hepatic microsomes. These cytochromes appear to be spectrally identical to those found in mammalian hepatic microsomes. Thus, the little skate, *Raja erinacea*, not only contains a hepatic mixed-function oxidase system that is similar to the analogous system found in mammals, but at least one of the components of the skate and mammalian systems appear to be spectrally identical. Attempts are now underway in our laboratory to purify the other enzymatic component of the skate mixed-function oxidase system, i.e., NADPH-cytochrome c reductase, and to reconstitute skate hydroxylase activity from soluble components.

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Response of Hepatic Microsomal Mixed-Function Oxidases in the Little Skate, *Raja erinacea*, and the Winter Flounder, *Pseudopleuronectes Americanus* to Pretreatment with TCDD (2,3,7,8-Tetrachlorodibenzo-p-Dioxin) or DBA (1,2,3,4-Dibenzanthracene)

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Experiments conducted in our laboratory last summer were designed to measure the response of mixed-function oxidases in little skate liver and kidney to exposure to chemicals which increase benzpyrene hydroxylase (AHH) activities in rats. Interpretation of the data was complicated by wide variability in data from both control and treated skates (*Bull MDIBL* 14, 7-12, 1974). We report here experiments which confirm, statistically, the increase of hepatic AHH activity in little skates and winter flounder after exposure to TCDD and DBA as compared with control activity. Statistical analyses using the

two-sided Mann-Whitney U test were performed by Dr. Joseph Haseman of the Biometry Branch at NIEHS

Little skates (500-1000 gm) were caught locally and stored in live cars. TCDD and DBA were administered interperitoneally. Winter flounder (ca. 250 gm) were caught locally and kept in aquaria with running sea water. Flounder were given oral doses of TCDD by stomach intubation; control fish received vehicle only, i.e. CO₂ oil: acetone (10:2). Microsomes were prepared from liver as previously described (*Bull. MDIBL*, 12, 12-15, 1974). Aniline hydroxylase, *d*-benzphetamine N-demethylase, benzpyrene hydroxylase (BPH) and 7-ethoxycoumarin O-deethylase (7-EC) activities were assayed as previously described (*Bull. MDIBL*, 12, 12-15, 1972; 13, 94-98, 1973). Hepatic microsomal cytochrome P-450 content was assayed at NIEHS by dithionite difference procedure (microsomes were prepared from frozen tissue). The dithionite difference spectra correct for hemoglobin contamination which can be large when frozen livers are used to prepare microsomes (*Bull. MDIBL*, 14, 7-11, 1974). In one case, microsomes were gassed with CO, frozen, and transported to NIEHS (packed in dry ice). Hemoglobin contamination of these microsomes was sufficiently low so that the carbon monoxide-reduced minus reduced spectra could be used for cytochrome P-450 assay (*J. Biol. Chem.*, 239, 2370-2378, 1964). Degradation of cytochrome P-450 to cytochrome P-420 was also less evident in spectra from microsomes prepared from fresh liver and transported frozen than spectra from microsomes prepared from frozen liver segments.

Table 1 shows the effect of orally administered TCDD on microsomal AHH and 7-EC activities in liver of winter flounder. 7-EC activity was increased 2-fold by animal treatment with TCDD. Although AHH activity did not show statistically significant increases after TCDD examination of the four control values revealed one which was very high. If this value were eliminated, significant increases (2-fold) in AHH activity of the TCDD-treated group would be evident. A striking feature of this data is the 10-fold higher AHH control values as compared with data collected from flounder in 1973, i.e., 2.54 ± 1.0 units/min/mg protein (*Bull. MDIBL*, 13, 94-98, 1973; *Drug Metab. Disp.*, 2, 545-555, 1974). Comparison of the AHH control values of little skate seen in Tables 2 and

Table 1

Effect of TCDD Administration on Two Microsomal Mixed-Function Oxidase Activities in Liver of Winter Flounder (*Pseudopleuronectes americanus*)

Enzyme Activity Assayed	Control Fish	TCDD Fish	P
Benzpyrene Hydroxylase ¹	36.7±12.8 (4) ² (17.3,26.6,28.6,74.3)	59.2±4.6 (6)	N.S.
7-Ethoxycoumarin O-Deethylase ³	.199±.068 (4)	.418 ±.070 (6)	<.05

¹Fluorescence Units formed/min/mg microsomal protein

²Mean ± SEM (N)

³Nmoles product formed/min/mg microsomal protein

Flounder were treated orally with 4.5 µg 2,3,7,8-tetrachlorodibenzo-p-dioxin/kg on days 1 and 3. Animals were sacrificed on day 8.

Table II
Effect of TCDD Administration on Mixed-Function Oxidase Activities and Cytochrome P-450
in Liver of Little Skate (*Raja erinacea*)

Parameter	Control	TCDD (Day 7) ¹	TCDD (Day 12) ¹
Benzpyrene Hydroxylase ²	1.22±0.17 (6)	18.4±3.0 (5) ⁵	22.7±5.5 (3) ⁵
7-Ethoxycoumarin O-Deethylase ³	0.360±0.058 (6)	0.563±0.096 (5)	0.743±0.225 (3)
Aniline Hydroxylase ³	0.30±0.06 (6)	0.58±0.15 (5)	0.51±0.20 (3)
d-Benzphetamine N-Demethylase ³	1.00±0.20 (6)	1.38±0.20 (5)	1.44±0.42 (3)
Cytochrome P-450 ⁴	0.24±0.02 (6)	0.25±0.03 (5)	0.34±0.07 (3)

¹Day upon which fish were killed after receiving intraperitoneal injections of 4.5 µg/kg tetrachlorodibenzo-p-dioxin in corn oil: acetone (10:2) on days 1 and 3.

²Fluorescence units formed/min/mg microsomal protein, mean ± SEM (N).

³NMoles product formed/min/mg microsomal protein mean ± SEM (N).

⁴NMoles/mg microsomal protein, mean ± SEM (N).

⁵Significantly different from control, P<0.05.

Table III
Effect of DBA Administration on Mixed-Function Oxidase Activities and Cytochrome P-450
in Liver of Little Skate (*Raja erinacea*)

Parameter	Control	DBA (Day 8) ¹	DBA (Day 13) ²
Benzpyrene Hydroxylase ³	0.61±0.19 (5)	5.04±2.26 (3) ⁶	7.91±3.87 (3) ⁷
7-Ethoxycoumarin O-Deethylase ⁴	1.18±0.24 (5)	2.30±0.31 (3)	2.70±0.25 (3) ⁷
Cytochrome P-450 ⁵	0.16±0.03 (3) ⁸	0.15±0.04 (3) ⁸	0.34±0.01 (3) ⁹

¹Day upon which fish were killed after receiving intraperitoneal injections of 10 mg/kg 1,2,3,4-dibenzanthracene in corn oil on days 1 and 3.

²Day fish killed after DBA, 10 mg/kg, on day 1.

³Fluorescence units formed/min/mg microsomal protein, mean ± SEM (N).

⁴Δ Fluorescent intensity/min/mg microsomal protein, mean ± SEM (N).

⁵NMoles/mg microsomal protein, mean ± SEM (N).

⁶Significantly different from control, P<0.07.

⁷Significantly different from control, P<0.04.

⁸Assayed in microsomes prepared from frozen liver.

⁹Assayed in microsomes frozen after isolation.

with those obtained in 1973 (0.17 ±0.10 units/min/mg protein) and in 1974 (0.11 ±0.04 units/min/mg protein) (*Bull. MDIBL*, 14, 7-12, 1974) also revealed an increase in activity in this species. Comparison of control metabolic activity towards the other substrates studied shows no change in the 1975 data from that obtained in previous years. These increases in enzyme activity, over time, suggest that near-shore and estuarine fish in Maine are being exposed to concentration of hydrocarbons, etc., high enough to cause partial induction of the liver system and that those levels of pollution are varying from year to year.

Ten-fold increases over control hepatic microsomal AHH activity in little skates which received TCDD intraperitoneally are shown in Table 2. Although increases in benzphetamine demethylase, 7-EC, and aniline hydroxylase

activities were also indicated, they were not statistically significant in these TCDD-treated skates. Hepatic microsomal cytochrome P-450 content was not changed by TCDD treatment of skates and no shift in the wavelength of the reduced P-450-CO complex absorbance peak was observed (from 450 nm to 448 nm, as occurs in rats).

With the potent but very toxic mfo inducer, TCDD, we were able to confirm increases of marine species' hepatic microsomal AHH activity in response to pre-treatment. However, we wished to use a safer compound for further characterization of this response in skates. 1,2,3,4-Dibenzanthracene (DBA), a polycyclic hydrocarbon, is relatively non-toxic to mammals (not included on *The Toxic Substances List*, 1974 edition, DHEW) and in mammals has been shown to be a relatively potent

inducer of mfo activity (*Mol. Pharmacol.*, 6, 178-183, 1970). However DBA apparently is relatively toxic to fish. Four out of seven skates which received 10 mg DBA/kg i.p. on days one and three, were dead by day 8, although four skates which received a single 10 mg DBA/kg i.p. dose survived to day 13 in a subsequent experiment. As shown in Table 3, both AHH and 7-EC activities were elevated in response to DBA pretreatment although cytochrome P-450 content was not increased and no shift in wavelength of the absorbance peak maximum of the reduced P-450-CO complex was observed.

These studies confirm that certain marine hepatic microsomal mixed-function oxidase activities can be increased by exposure to polycyclic hydrocarbons and TCDD without a corresponding change in the reduced cytochrome P-450-CO complex spectra or P-450 content. 1,2,3,4-Dibenzanthracene was identified as a relatively non-toxic (to mammalian species) inducer suitable for use in characterizing the induced AHH of little skates.

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Length-Tension Relationships in *Echinarachnius parma* Cleavage Furrows

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The division mechanism of echinoderm eggs consists of an equatorial surface ring underlain by microfilaments. The ring actively contracts, constricting the spherical egg into two blastomeres. The cross sectional area of the contractile ring remains relatively constant during cytokinesis so that it literally disappears as it functions and vanishes as cleavage is completed. Simple periodic measurements of equatorial diameter reveal that the circumference decreases at 45 per minute during the first cleavage and at 30 per minute during the second.

The force exerted by the furrow can be measured directly by placing a calibrated, flexible needle through and at right angles to the equatorial plane so that the cleavage process remains incomplete, because the furrow cannot overcome the resistance to deformation of the needle. When the furrow contracts isometrically, it consists of 2 parallel contractile strips of a length determined by the distance between the calibrated needle and a second, rigid needle which is also inserted across the division plane (Rappaport, *Science* 156, 1241, 1967). As the contraction approaches the isometric point, the shortening rate of the circumference appears somewhat erratic, because it is characterized by frequent pauses and small reversals of progress. The force exerted by the fi. cleavage furrow averaged 1.25×10^{-3} dyne and that o. the second averaged 1.48×10^{-3} d. At second cleavage the two blastomeres normally divide synchronously. Measurements made on one of a pair reveal that the contractile mechanism of the measured cell can continue to exert maximum tension up to 9 minutes after the control cell completed division. This observation suggests that the disappearance of the contractile ring may be intimately related to its function. Paired measurements of tensions exerted by the same furrow in isometric contraction at 2 different lengths, the shorter of which was about 30% less than the longer, were not

significantly different. The forces measured averaged 1.2×10^{-3} d. for the shorter, and 1.30×10^{-3} d. for the longer (Supported by NSF Grant BMS 74-18380)

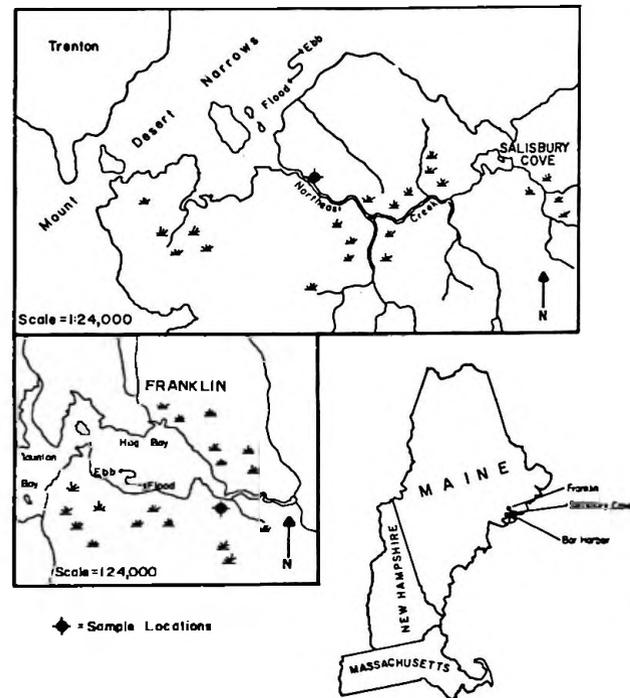
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Primary Production and Substrate Selective Properties of Maine Salt Marsh Plants

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Coastal marshes are among the most productive natural or cultivated ecosystems. Since there is increasing pressure to manage the economically important coastal zone, a knowledge of the component ecosystems is essential. The second year's study of the aerial primary productivity and underground biomass dynamics in Maine salt marshes was conducted simultaneously with studies

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in Delaware and Georgia. Substrate plant interaction were evaluated through the use of nutrient enrichment and soil analysis experiments.

Primary production was measured using paired plot harvest techniques (Wiegert and Evans, *Ecology* 45: 49-63, 1964); and underground plant parts and soil were collected by coring (Gallagher, *Soil Sci. Soc. Proc.* 36: 154-155). Stands of plants in Northeast Creek and Hog Bay marshes (Fig. 1) were sampled at 8 week intervals from April to October. Plant analysis procedures are explained in detail by Reimold *et al.* (*Recent Advances in Estuarine Research*, in press). Nutrient enrichment and soil analysis procedures were similar to those used by Gallagher (*Am. J. Bot.*, 62: 644-648, 1975).