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Uptake, Metabolism and Discharge of Polycyclic Aromatic Hydrocarbons by Marine Fish

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Abstract

The uptake, metabolism and discharge of two polycyclic aromatic hydrocarbons, ^{14}C-naphthalene and ^{3}H-3,4-benzopyrene, were studied in 3 species of marine fish (mudsucker or sand goby, *Gillichthys mirabilis*; sculpin, *Oligocottus maculosus*; sand dab, *Citharichthys stigmaeus*). The path of hydrocarbons through the fish included entrance through the gills, metabolism by the liver, transfer of hydrocarbons and their metabolites to the bile, and, finally, excretion. The gall bladder was a major storage site of labeled hydrocarbons and their metabolites. The major product of ^{3}H-3,4-benzopyrene metabolism was tentatively identified as 7,8-dihydro-7,8-dihydroxybenzopyrene. The ^{14}C-naphthalene was metabolized to 1,2-dihydro-1,2-dihydroxynaphthalene after 24 h exposure. The urine appeared to be the major avenue for discharge of labeled hydrocarbon from the body. Our laboratory results indicated that certain polycyclic aromatic hydrocarbons were rapidly taken up from seawater by the above fish, but detoxification mechanisms existed for efficient removal of these compounds from their body tissues.

Introduction

In recent papers, we have presented our results on the uptake of petroleum hydrocarbons by marine invertebrates (Lee et al., 1972a, b). Neither straight chain nor aromatic hydrocarbons were metabolized by these invertebrate systems. Vertebrates, including fish, are able to metabolize various types of hydrocarbons (Diamond and Clark, 1970; Clark and Diamond, 1971). The present report is concerned with the uptake, metabolism, and discharge of two polycyclic aromatic hydrocarbons, ^{14}C-naphthalene and ^{3}H-3,4-benzopyrene, by three species of marine fish (mudsucker or sand goby, *Gillichthys mirabilis*; sculpin, *Oligocottus maculosus*; sand dab; *Citharichthys stigmaeus*). The polycyclic aromatic hydrocarbons were used because of their toxic and carcinogenic properties (Nelson-Smith, 1970).

Metabolism of polycyclic aromatic hydrocarbons by mammals has been reported (Boyland and Solomon, 1955; Sims, 1967; Jerina et al., 1970; Robertson and Dunstan, 1971), but the fate of these hydrocarbons in other vertebrates is largely unknown. Embryonic gonad tissue from trout has been shown to degrade benzopyrene to unidentified water-soluble derivatives (Clark and Diamond, 1971), while trout liver was able to hydroxylate biphenyl (Creaven et al., 1965; Adamson, 1967).

Both naphthalene and benzopyrene are found in petroleum, and thousands of kilograms of these compounds undoubtedly enter the sea each year. Benzopyrene is a well known carcinogen in vertebrates (Haddow, 1958; Heidelberger, 1964), and is a minor component of crude petroleum. After combustion of petroleum, however, there is a large increase in the amount of benzopyrene. Marine fish and invertebrates accumulated benzopyrene in polluted water (Shimkin et al., 1951; Mallet and Priou, 1967; review: Zobell, 1971). The marine copepod *Calanus helgolandicus* was killed by benzopyrene at concentrations of 4 parts per billion (Lee, unpublished data).

Material and Methods

Mudsuckers (*Gillichthys mirabilis*) were obtained from a local bait shop and kept in a plastic bucket until used for experiments. The tidepool sculpins *Oligocottus maculosus* were collected at low tide from rocky pools near La Jolla, California (USA). Sand dabs, *Citharichthys stigmaeus*, were caught in an estuary near Tiajuana, Mexico. Sand dabs were held without feeding in ceramic tanks in fresh running seawater. All experiments were completed within 2 weeks after capture of the fish.

For uptake experiments, *Gillichthys mirabilis* and *Oligocottus maculosus* were placed in 2 l beakers which contained either ^{14}C-naphthalene (15.6 μci/mg) or ^{3}H-3,4-benzopyrene (44 mci/mg) in 1 l seawater. The naphthalene and benzopyrene were dissolved in 10 μl of benzene before addition to the seawater. The quantities of naphthalene and benzopyrene used were soluble in water (Bohon and Clausen, 1951). Only 1 fish was placed in each beaker and, at specified times, 3 to 5 fish were taken out and rinsed with methanol, followed by dissection and removal of tissues. For discharge experiments, fish were transferred to seawater free of hydrocarbon, after various periods of exposure to the hydrocarbon. The methanol rinse was to remove adsorbed radioactivity, and radioactivity remaining after this rinse was considered to be hydrocarbon which had been taken up. Tissues, after weighing, were extracted with 2.5 ml of chloroform:

Table 1. *Gillichthys mirabilis. Uptake of ^{3}H-3,4-benzopyrene. Each fish was placed in a 2 l beaker containing 1 µg (84 × 10^6 cpm) of ^{3}H-3,4-benzopyrene in 1 l seawater. Uptake, in µg 3,4-benzopyrene per 100 g dry weight of tissue, is given in normal print, followed by range of values observed for 4 fish in parentheses. Total activity taken up in each tissue, in thousands of counts per min (cpm), is given below in italics followed by standard deviation, σ, in parentheses*

Time (h)	Tissue					
	Liver	Gut	Gill	Flesh	Gall bladder	Heart
0.25	2 (1—3) *17 (σ, 2)*	1 (1—2) *4.5 (σ, 0.7)*	14 (10—19) *31 (σ, 5)*	1 (1—2) *1.7 (σ, 0.6)*	0.1 *0.3*	4 (2—9) *0.35 (σ, 1.2)*
0.50	9 (7—10) *30 (σ, 4)*	2 (1—4) *6.8 (σ, 0.9)*	30 (27—31) *66 (σ, 5.6)*	2 (1—3) *3 (σ, 0.5)*	1 *0.4 (σ, 0.1)*	9 (6—10) *0.78 (σ, 0.27)*
1.0	7 (6—10) *42 (σ, 1.1)*	3 (2—6) *7.5 (σ, 0.9)*	33 (27—40) *73 (σ, 8)*	2 *4 (σ, 0.2)*	4 (2—5) *2.2 (σ, 0.37)*	8 (5—12) *0.9 (σ, 0.3)*
25	8 (6—9) *43 (σ, 2)*	8 (5—10) *16 (σ, 3)*	20 (15—27) *49 (σ, 2.8)*	3 (2—5) *4.6 (σ, 1.4)*	121 (110—132) *190 (σ, 3.0)*	
96	10 (7—13) *47 (σ, 5.7)*	18 (12—22) *36 (σ, 2.2)*	29 (25—33) *47 (σ, 7)*	2 (2—3) *4.8 (σ, 0.35)*	740 (720—750) *1,300 (σ, 110)*	30 (27—31) *5.2 (σ, 0.5)*

methanol (2:1 v/v), and a 50 µl aliquot was put into 15 ml of Aquasol (New England Nuclear) and counted in a liquid scintillation counter (Beckman DPM-100). The results are reported as counts per minute (cpm) and as hydrocarbon taken up (in micrograms) per unit dry weight of tissue (per gram for naphthalene experiments and per 100 g for benzopyrene experiments). We attempted to use fish of approximately the same weight for all experiments (0.54 g was average dry weight for *G. mirabilis*, 0.75 g average dry weight for *O. maculosus*). The tissues used were the liver, gut (including intestine and stomach), gill, flesh, gall bladder, and heart. A small section of the flesh (approximately 1/5 of the whole) was taken for extraction.

For uptake experiments by young sand dabs (*Citharichthys stigmaeus*, approximate dry weight 5 g), fish were placed in 3 l glass aquariums which contained 1.5 l of seawater over a sand bottom. Known weights of ^{14}C-naphthalene or ^{3}H-3,4-benzopyrene were dissolved in benzene and added to the seawater after the fish had been in the aquarium for 24 h. Exposures to hydrocarbon were for 1 h, followed by transfer to clean seawater. At various intervals, urine samples were taken and the urine sample was added to 15 ml of Aquasol and counted in a liquid scintillation counter. For the collection of urine, fish were removed from the aquarium and placed in clean enamel pans. The urinary papilla area was dried well using absorbant tissue. The urine was then forcibly expressed from the bladder by gentle finger pressure exerted on the exterior abdominal region overlying the bladder. The muscle spincter of the urinary opening was thus forced open and urine expelled. As the urine formed a small liquid drop above the papilla, it was drawn into a 50 µl syringe. There was no flow of urine over the body of the fish.

In order to identify hydrocarbon metabolities, lipid extracts were concentrated under nitrogen and applied to silicic acid thin-layer plates. The plates were developed in benzene:petroleum ether (1:1 v/v) or chloroform. The mobility (R_f) value of the radioactive compounds on the plates was determined by autoradiography, using Single Coated Blue Sensitive X-ray film (Eastman Kodak Co.). The reported R_f values of the various metabolities of benzopyrene and naphthalene were used in tentatively identifying unknown compounds (Sims, 1967; Jerina et al., 1970).

Several fish from an oil-polluted harbor (San Diego, California) were analyzed for polycyclic aromatic hydrocarbons. Lipid extracts were passed through a silicic acid column, and elution with petroleum ether gave a hydrocarbon fraction. This hydrocarbon fraction was applied to silicic acid thin-layer plates and run in petroleum ether:benzene (1:1 v/v). Polycyclic aromatic hydrocarbons were visualized by iodine, eluted with ethanol, and a spectrum of the hydrocarbons was taken (Cary Spectrophotometer) between 260 and 360 nm. The spectrum obtained was compared with those of authentic standards.

Results

Uptake

The uptake of ^{3}H-3,4-benzopyrene and ^{14}C-naphthalene was rapid, and these compounds could be detected in most tissues within a few minutes after introduction of the hydrocarbon (Tables 1, 2, 4, 6, 7). In Table 1, the standard deviation and the range of values are given for ^{3}H-3,4-benzopyrene uptake experiments; 4 fish were taken in each time period and each fish was separately analyzed. Only the mean values are given in the other experiments, but a similar range of values was noted.

Table 2. *Oligocottus maculosus. Uptake of ^{3}H-3,4-benzopyrene. Each fish was placed in a 2 l beaker containing 1 µg ^{3}H-3,4-benzopyrene (84×10^{6} cpm) in 1 l seawater. Results are expressed in µg benzopyrene per 100 g dry weight of tissue, normal print. Total activity in each tissue, in thousands of cpm, is given in italics below specific activities*

Time (h)	Tissue				
	Liver	Gut	Gill	Flesh	Heart
0.25	2	2	1	1	1
	9	*4.7*	*0.30*	*0.16*	*0.50*
0.50	4	6	10	8	3
	12	*9.7*	*2.4*	*1.1*	*0.70*
1.0	12	16	20	13	7
	22	*14*	*4.7*	*1.6*	*1.2*

take by the liver was probably limited by its rate of metabolism. However, the amount of ^{14}C-naphthalene taken up by the liver of *G. mirabilis* continued to increase even after 2 h exposure (Table 5); therefore, the liver can deal with higher quantities of naphthalene than benzopyrene.

All three species of fish took up more naphthalene than benzopyrene. An increase in concentration of benzopyrene in the water from 1 to 6 µg did not result in further uptake (Tables 1 and 4). An increase in the amount of naphthalene in the water from 32 µg to 29 mg resulted in a large increase in total naphthalene uptake (Tables 5 and 6).

Uptake of benzopyrene by the various tissues, except for the gall bladder, did not increase after 24 h exposure (Table 1). The maximum amount of benzo-

Table 3. *Gillichthys mirabilis. Uptake and discharge of ^{3}H-3,4-benzopyrene. Each fish was placed in a 2 l beaker containing 1 µg (84×10^{6} cpm) of ^{3}H-3,4-benzopyrene in 1 l seawater. Results are expressed in µg benzopyrene per 100 g dry weight of tissue, normal print. Total activity fixed, in each tissue, in thousands of cpm, is given in italics below specific activities. Group A: After 1 h fish were transferred to seawater free of hydrocarbons; Group B: after 2 h fish were transferred to seawater free of hydrocarbons; Group C: after 4 h fish were transferred to seawater free of hydrocarbons*

Group	Time (h)	Tissue					
		Liver	Gut	Gill	Flesh	Gall bladder	Heart
A	1	7	3	33	2	4	8
		42	*7.5*	*73*	*4*	*2.2*	*0.9*
	25	5	3	1	3	190	2
		28	*10*	*1.7*	*3.6*	*107*	*0.26*
B	2	8	10	25	2	4	12
		45	*17*	*65*	*3.9*	*1.7*	*1.2*
	122	1	2	1	0.5	630	—
		3.7	*5.3*	*1.1*	*0.4*	*340*	*25*
C	4	10	24	20	3	3	
		49	*35*	*59*	*4*	*4.3*	
	28	9	5	2	1	1	
		24	*17*	*2.9*	*3.2*	*340*	

In the first hour of the uptake experiments, the liver, gut, and gill tissues contained most of the hydrocarbon (Tables 1—8). Longer periods of exposure resulted in accumulation of hydrocarbon by the flesh and especially by the gall bladder (Tables 1, 3, 5, 7). For the various uptake and discharge experiments, a portion of the radioactivity is due to hydrocarbon metabolities, as will be discussed later.

In ^{3}H-3,4-benzopyrene uptake experiments, the liver of *Gillichthys mirabilis* showed a very steady increase in radioactivity for the first hour, but no further increase in radioactivity was noted during the next 95 h (Tables 1, 3, 4). Thus, a steady state is reached within 1 h, so that benzopyrene entering is balanced by the amount of benzopyrene and its metabolites leaving the liver. Thus, the rate of benzopyrene uptake and its metabolites taken up was 5×10^{-4} µg for the liver, 3×10^{-4} µg for the gut, 6×10^{-4} µg for the gills, 3×10^{-4} µg for the flesh, 0.6×10^{-4} µg for the heart, and 1.6×10^{-2} µg for the gall bladder. Maximum uptake for naphthalene was not determined.

The path of uptake would appear to be through the gills, followed by accumulation of hydrocarbon and its metabolites by the liver, gut, and flesh. The gall bladder was the final storage site.

Several fish, including anchovies and smelt, were collected from San Diego Bay, which is a large shipping port. Analysis for polycyclic aromatic hydrocarbons revealed up to 10 µg of benzopyrene per fish (dry weight of fish varied from 2 to 10 g) with lesser amounts of several other unidentified aromatic hydrocarbons.

Table 4. *Gillichthys mirabilis. Uptake and discharge of ^{3}H-3,4-benzopyrene. Each fish was placed in a 2 l beaker which contained 6 µg (89 × 10^{6} cpm) of ^{3}H-3,4-benzopyrene in 1 l seawater. Results are expressed in µg benzopyrene per 100 g dry weight, normal print. Total activities in each tissue are given in italics below specific activity. After 1 h exposure, fish were transferred to seawater free of hydrocarbons*

Time (h)	Tissue					
	Liver	Gut	Gill	Flesh	Gall bladder	Heart
0.25	1	1	8	2	1	1
	700	*1200*	*2300*	*800*	*100*	*10*
0.50	5	2	8	4	3	2
	2300	*5600*	*2300*	*1300*	*200*	*20*
1.0	8	3	15	5	5	1
	7600	*4900*	*3900*	*1800*	*700*	*10*
1.5	7	4	10	5	21	1
	7100	*3800*	*3700*	*1100*	*3800*	*30*
2	5	5	8	4		
	2500	*3000*	*1300*	*1400*		
3	5	2	4	1	30	2
	2200	*1000*	*1400*	*700*	*8500*	*20*
25	5	3	3	2	210	
	2600	*1300*	*900*	*800*	*30,000*	

Discharge

After various periods of exposure to the labeled hydrocarbons, fish were transferred to seawater free of hydrocarbon. The radioactivity in the various tissues dropped rapidly, so that after 24 h in clean seawater, more than 90% of the radioactivity taken up had been lost from most of the fish tissues in the ^{14}C-naphthalene experiments (Tables 5, 6, 7). Exposure for several hours to ^{3}H-3,4-benzopyrene, followed by transfer to clean seawater for 24 h, resulted in the liver, gut, gill, and flesh losing 50, 50, 90, and 20% of their radioactivity, respectively (Tables 3 and 4). Thus, the fish could flush out naphthalene and its metabolites at a greater rate than benzopyrene.

A notable exception to the decrease of radioactivity of tissues was the large increase in radioactivity in the gall bladder after transfer to clean seawater. Thus, it appeared that radioactivity lost in the various tissues during the discharge experiments was transferred to the bile. Fish exposed to ^{14}C-naphthalene and then transferred to clean seawater showed an increase in radioactivity in the gall bladder but, after 24 h in clean seawater, gall bladder radioactivity was also reduced (Table 5). On the other hand, the discharge experiments with ^{3}H-3,4-benzopyrene showed storage of radioactivity in the gall bladder, but no loss of gall bladder radioactivity after 24 h (Table 3).

To observe excretion of hydrocarbons and their metabolites, samples of urine were collected from the flatfish *Cithatichthys stigmaeus* after 1 h exposure of labeled hydrocarbon. After 20 h, the urine was highly radioactive in both ^{14}C-naphthalene and ^{3}H-3,4-benzopyrene experiments (Tables 8 and 9). The feces were not analyzed.

Careful examination of the fish revealed no tumors in the various tissues exposed to benzopyrene. Of course, the experiments were of short duration (less than 4 days), and tumor development would not be expected to occur in such a short time.

Metabolism

To determine the fate of the radioactive hydrocarbons during the experiments, lipid extracts of the various fish tissues were applied to thin-layer plates. Radioautographs of these plates were used to determine the R_f values of the original hydrocarbon and its metabolites. After 30 min exposure to ^{3}H-benzopyrene or ^{14}C-naphthalene, hydroxylated derivatives were detected in the liver and gut, but the gall bladder showed only the original hydrocarbon. Approximately 10% of the benzopyrene had been converted to 6-hydroxybenzopyrene, while 7% of the naphthalene was oxidized to hydroxynaphthalene. Exposure of fish to hydrocarbons for 24 h resulted in accumulation of hydroxylated derivatives in the gall bladder. Only 10% of the original benzopyrene remained in the various tissues after 96 h exposure to ^{3}H-3,4-benzopyrene. The major radioactive compound in all tissues was a compound tentatively identified as 7, 8-dihydro-7,8-dihydroxybenzopyrene.

In the ^{14}C-naphthalene experiment, after 48 h exposure, the gall bladder showed 4 compounds: naphthalene, hydroxynaphthalene, 1,2-dihydro-12-dihydroxynaphthalene, and possibly either a glutathione or glycoside conjugate of 1,2-dihydro-1,2-dihydroxynaphthalene. In all tissues the major compound after 48 h of exposure was 1,2-dihydro-1,2-dihydroxynaphthalene.

Table 5. *Gillichthys mirabilis. Uptake and discharge of ^{14}C-naphthalene. Each fish was placed in a 2 l beaker containing 32 µg (850 × 10^3 cpm) of ^{14}C-naphthalene. Results are expressed in µg naphthalene/g dry weight, normal print. Total activity in each tissue is given in italics below specific activities. Group A: After 1 h exposure, fish were transferred to seawater free of hydrocarbons; Group B: after 2 h exposure, fish were transferred to seawater free of hydrocarbons*

Group	Time (h)	Tissue					
		Liver	Gut	Gill	Flesh	Gall bladder	Heart
A	1.0	14.1	1.9	2.7	1.1	1.9	4.8
		65000	*3600*	*2100*	*1500*	*300*	*400*
	25	0.8	0.7	0.1	0.1	5.7	2.6
		500	*400*	*30*	*10*	*1700*	*70*
B	0.25	1.0	0.5	0.5	0.8	0.2	—
		3900	*1000*	*500*	*400*	*60*	*0*
	0.50	5.8	3.6	4.8	0.6	2.1	2.3
		21000	*5000*	*3800*	*200*	*400*	*100*
	1.0	14.1	1.9	2.7	1.1	1.9	4.8
		65000	*3600*	*2100*	*1500*	*300*	*400*
	2.0	40.6	5.6	6.0	1.9	4.2	6.7
		112000	*5100*	*4100*	*1600*	*800*	*350*
	3.0	37.4	2.2	4.0	1.1	7.9	1.1
		165000	*3700*	*3000*	*1100*	*6000*	*100*
	26.0	1.2	0.1	0.2	0.2	5.5	1.1
		6300	*260*	*120*	*200*	*900*	*40*
	140	0.2	0.4	0.1	0.2	6.9	0.9
		300	*500*	*70*	*300*	*1100*	*50*

Table 6. *Gillichthys mirabilis. Uptake and discharge of ^{14}C-naphthalene. Each fish was placed in a 2 l beaker which contained 29 mg (850 × 10^3 cpm) ^{14}C-naphthalene in 1 l seawater. After 1 h exposure, fish were transferred to seawater free of hydrocarbon. Results are expressed in µg naphthalene/g dry weight of tissue, normal print. Total activity fix of each tissue in cpm is given in italics below specific activity*

Time (h)	Tissue					
	Liver	Gut	Gill	Flesh	Gall bladder	Heart
0.25	400	300	1900	800	600	500
	2000	*1100*	*300*	*170*	*140*	*30*
0.50	700	1500	1700	1300	1600	200
	2800	*600*	*470*	*200*	*310*	*20*
1.0	2200	5600	9000	9100	4200	2500
	11500	*2500*	*2300*	*1600*	*600*	*300*
1.5	2000	4200	5900	3500	5700	
	7300	*1400*	*1200*	*800*	*730*	
2	2300	1500	2300	3600	11000	
	9400	*400*	*800*	*800*	*2200*	
3	1100	1200	2100	3000	15000	1700
	4000	*600*	*300*	*600*	*2500*	*200*
25	150	1600	200	70	20000	800
	600	*580*	*10*	*10*	*2200*	*70*

Table 7. *Oligocottus maculosus. Uptake and discharge of ^{14}C-naphthalene. Uptake values are reported as µg/g dry weight of tissue normal print, and in total activity in thousands of cpm in the whole tissue, italics. Group A: Fish transferred to seawater free of hydrocarbons after 1 h exposure; Group B: fish transferred to seawater free of hydrocarbons after 2 h exposure; Group C: fish transferred to seawater free of hydrocarbons after 3 h exposure*

Group	Time (h)	Tissue					
		Liver	Gut	Gill	Flesh	Gall bladder	Heart
A	0.25	3.0	1.5	2.0	1.7	1.5	0.5
		1.5	*1.9*	*0.42*	*0.30*	*0.21*	*0.05*
	0.50	21	4.9	4.4	3.0	2.7	1.6
		12	*3.7*	*1.2*	*0.70*	*0.20*	*0.09*
	1.0	38	7.2	6.3	8.1	2.2	4.1
		16	*4.1*	*2.6*	*1.5*	*0.27*	*0.22*
	2.0	2.2	2.4	2.8	1.7	3.5	3.6
		1.9	*1.8*	*0.50*	*0.25*	*0.38*	*0.11*
	25	1.2	0.9	0.1	0.6	2.1	0.6
		0.40	*0.95*	*0.10*	*0.12*	*0.20*	*0.02*
B	2.0	26	18	19	5.1	2.8	20.1
		16	*13*	*16*	*1.2*	*0.30*	*1.5*
	4.0	21	15	5.3	2.9		
		22	*10*	*3.6*	*1.1*		
	50	3.2	0.5	3.9	0.8		
		2.1	*0.60*	*0.60*	*0.40*		
C	3.0	40	81	18	51	6.2	13
		23	*37*	*11*	*10*	*0.70*	*0.70*
	73	0.1	1.0	1.6	1.0	14	—
		0.25	*0.67*	*0.40*	*0.12*	*1.1*	*0*

Table 8. *Citharichthys stigmaeus. Uptake of ^{3}H-3,4-benzopyrene. Fish were placed in 4 l aquaria containing 2 µg (168×10^6 cpm) of ^{3}H-3,4-benzopyrene in 1.5 l sea water for 1 h. Results are expressed in µg benzopyrene per 100 g dry weight of tissue*

Tissue	Counts per minute (total)	Benzopyrene (µg/100 g)
Liver	530,000	13
Gut	117,000	1
Gill	400,000	40
Flesh	25,000	3
Gall bladder	59,000	9
Heart	17,000	15

After 1 h exposure to ^{3}H-3,4-benzopyrene, fish were transferred to fresh seawater free of hydrocarbons, and urine samples were taken at various times. Results are given in µg benzopyrene per ml urine

Time (h)	Counts per minute	Urine volume (µl)	Benzopyrene (µg/ml)
1	0	20	—
20	430,000	30	0.33
40	400,000	20	0.46

Table 9. *Citharichthys stigmaeus. Uptake of ^{14}C-naphthalene. Fish were placed in 4 l aquaria containing 32 µg (850×10^3 cpm) of ^{14}C-naphthalene in 1.5 l seawater for 1 h*

Tissue	Counts per minute (total)	Naphthalene per dry weight tissue (µg/g)
Liver	21,000	14.2
Gut	13,000	2.4
Gill	5,400	17.1
Flesh	4,700	8.2
Gall bladder	1,900	3.4
Heart	1,200	3.9

After 1 h exposure, fish were transferred to seawater free of hydrocarbons and urine samples were taken at various times

Time (h)	Counts per minute	Amount of urine (µl)	Naphthalene per urine sample (µg/ml)
Experiment 1			
1	0	10	—
4	0	13	—
6	430	24	0.5
27	870	18	1.6
52	340	13	0.7
Experiment 2			
1	0	10	—
4	0	7	—
6	0	19	—
27	680	21	1.2
52	81	10	0.3

Lipid extracts of the urine from ^{3}H-3,4-benzopyrene experiments showed a compound, which after mild acid treatment, had properties similar to 7,8-dihydro-7,8-dihydroxybenzopyrene. The original compound in the urine was probably dihydroxybenzopyrene conjugated with either sulfate or sugar. The products of ^{14}C-naphthalene metabolism in the urine were not analyzed. Boyland and Solomon (1955) isolated 1,2-dihydro-1-naphthyl glucosiduronic acid from the urine of rats given naphthalene.

Discussion

The rapid uptake, metabolism, and discharge of benzopyrene and naphthalene by marine fish was similar to the results obtained with mammals. In the work of Falk and co-workers (Kotin et al., 1959; Falk et al., 1962) ^{14}C-benzopyrene was injected intravenously into a rat. The liver exhibited a rapid buildup of radioactivity, followed in a few hours by reduction in amount of radioactivity in the liver and its transfer to the bile, and, finally, excretion in the feces. Most of the original radioactivity was recovered in the feces. Very little radioactivity was found in the urine, kidney, and stomach. Rat liver homogenates metabolized benzopyrene to 3-hydroxy-benzopyrene, benzopyrene 1,6,2,6-quinones, 1,2-dihydro-1,2-dihydroxybenzopyrene and 7,8-dihydro-7,8-dihydroxybenzopyrene (Sims, 1967).

When ^{3}H-3,4-benzopyrene was given to fish, there was buildup in the liver with later transfer or radioactivity to the gall bladder (Tables 1, 3, 4). The gills were assumed to be the site of hydrocarbon entrance, with a micellar layer which adsorbs the hydrocarbons and then passes the hydrocarbons to other tissues. Although the feces were not monitored, it appeared from the amount of radioactivity in the urine that the urine was the main avenue through which benzopyrene metabolites were excreted from the fish. The products of benzopyrene metabolism in the fish were similar to rat metabolites, with the main product being a compound with properties similar to 7,8-dihydro-7,8-dihydroxybenzopyrene.

There are at least two paths by which fish can take up hydrocarbon. The first path is with the food, where hydrocarbon entrance is via the gut. The second path is dealt with in this paper, namely, uptake of hydrocarbon dissolved in the water, where entrance is through the gills. Since the gut and gill are very different tissues, it would be expected that transfer and retention of hydrocarbon would be quite different for the two paths of uptake.

The microsomes of rat liver metabolized naphthalene to 1-hydroxynaphthalene, trans-1,2-dihydro-1,2-dihydroxynaphthalene, and S-(1,2-dihydro-2-hydroxyl-naphthyl) glutathione (Jerina et al., 1970). In fish, the main product of naphthalene metabolism was 1,2-dihydro-1,2-dihydroxynaphthalene. The reason why fish are able to metabolize much larger quantities of naphthalene than benzopyrene is not clear (Tables 1, 4, 5, 6). Possibly benzopyrene causes a diminution of the metabolic rate so that less hydrocarbon is taken up. The gradual buildup of radioactivity in the gall bladder of the fish after introduction of labeled hydrocarbon is of interest in the light of many investigations, which report that lipid-soluble foreign compounds injected into mammals are excreted in the bile (Kotin et al., 1959; Falk et al., 1962; Ostrow, 1967; Wright and Diamond, 1969; Abdel Aziz et al., 1971; Barrow and Griffiths, 1971; Gingell and Bridges, 1971; Johnson et al., 1971).

The introduction into mammals of lipid-soluble foreign compounds causes activation of various microsomal enzymes in the liver (Gram and Fouts, 1968; Remmer et al., 1968; Spencer and Fischer, 1971). The function of these enzymes is to detoxify the foreign compounds by hydroxylation and conjugation with water-soluble moieties such as sulfate or glucose. The compounds then enter the bile and are excreted. This pathway of detoxification of various lipid-soluble compounds by making them water soluble appears to be utilized by fish and sharks (Adamson, 1967). From the data presented here, it is evident that enzymes for dealing with polycyclic aromatic hydrocarbons are highly active in the livers of marine fish.

A side note to this discussion is the fact that, in preliminary experiments, we found that ^{14}C-DDT was not hydroxylated or metabolized by the fish. There was storage of ^{14}C-DDT in the liver, but no transfer of radioactivity to the gall bladder. Fish liver extracts were unable to hydroxylate ^{13}C-DDT, whereas ^{14}C-naphthalene and ^{3}H-3,4-benzopyrene were rapidly hydroxylated by the same extracts. Thus, halogens on the aromatic rings prevent metabolism.

Our results indicate that certain polycyclic aromatic hydrocarbons can be rapidly taken up from seawater by fish, but detoxification mechanisms exist which allow efficient removal of these compounds from the body tissues.

Summary

1. Three species of marine fish (mudsucker or sand goby, *Gillichthys mirabilis*; sculpin, *Oligocottus maculosus*; sand dab, *Citharichthys stigmaeus*) were exposed to the polycyclic aromatic hydrocarbons ^{14}C-naphthalene and ^{3}H-3,4-benzopyrene, for various periods of time.

2. Within minutes, all 3 fish species rapidly took up labeled hydrocarbons through the gills. Radioactivity then built up in the liver, where metabolism of the hydrocarbon took place, followed by transfer of hydrocarbon and its metabolites of the bile, and finally excretion of the metabolites. More naphthalene could be taken up by the fish than benzopyrene. The radioactivity of the liver rose during the first few hours of the uptake experiments, and then leveled

off. Further uptake by the liver was probably limited by the rate of hydrocarbon metabolism.

3. The major metabolite of ^{3}H-3,4-benzopyrene was tentatively identified as ^{3}H-7,8-dihydro-7,8-dihydroxybenzopyrene, while the main product of ^{14}C-naphthalene metabolism was ^{14}C-1,2-dihydro-1,2-dihydroxynaphthalene.

4. The gall bladder was a major storage site for the hydrocarbons and their metabolities, while urine was an important avenue for the excretion of the water-soluble metabolites.

5. When fish were exposed to ^{14}C-naphthalene for several hours and then transferred to clean seawater for 24 h, the radioactivity of the various tissues was reduced ten-fold. The same type of discharge experiment repeated with ^{3}H-3,4-benzopyrene resulted in the liver, gut, gill, and flesh losing 50, 50, 90 and 20% of their radioactivity, respectively. Thus, the fish could flush out naphthalene and its metabolites at a greater rate than benzopyrene and its metabolites.

6. A detoxification mechanism, which was found in 3 species of marine fish, allowed efficient removal of polycyclic aromatic hydrocarbons from the body tissues.

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