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Compartmentalization of Ingested Labelled Petroleum in Tissues and Bile of the American Eel (*Anguilla rostrata*)

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The fate of petroleum hydrocarbons has been described in a variety of marine animals (VARANASI & MALINS 1977); however, there is not enough detailed information on the compartmentalization and metabolic fate of petroleum in fish species, and few studies have examined the uptake and clearance of ingested petroleum hydrocarbons.

Although fish readily take up hydrocarbons from the environment, substantial and rapid decreases in tissue oil concentrations occur once the animals are placed into clean water. This indicates the existence of a metabolic capacity for the removal of these lipophilic compounds from body tissues, shown in several species of fish (LEE et al. 1972, VARANASI et al. 1978).

High levels of hydrocarbons are found in the bile of fish after exposure, and appear to be mainly due to the presence of metabolic products (ROUBAL et al. 1977, MELANCON & LECH 1978). Liver has also been reported to accumulate significant levels of petroleum hydrocarbons (LEE et al. 1972, KORN et al. 1976), including metabolic products (ROUBAL et al. 1977). This finding is consistent with the known role of the liver in the metabolism of petroleum hydrocarbons.

The present study was undertaken to evaluate the dynamics of uptake and clearance, as well as tissue distribution, of petroleum hydrocarbons in the American eel, Anguilla rostrata, following oral exposure to crude oil. Such analysis of hydrocarbon distribution and their release from tissues and body fluids may add to an understanding of the mechanisms by which fish clear petroleum hydrocarbons from their systems. Insofar as the metabolism of hydrocarbons results mainly in the conversion of the lipophilic compounds into water-soluble metabolites which can be excreted (ADAMSON 1967), a simple two-phase partitioning technique based on this principle was used to extract hydrocarbons from tissues and body fluids.

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MATERIALS AND METHODS

Immature American eels (A. rostrata LeSueur) of weights ranging from 70-100 g were used. They were obtained from the St.Lawrence River at Cornwall, Ontario, and held in the laboratory in a flow-through system using dechlorinated Ottawa city tap water. Temperature ranged from 8 to 15°C, winter to summer, respectively. A photoperiod of 12L:12D (fluorescent light) was used, and the eels were allowed to acclimate to laboratory holding conditions for at least 3 to 4 weeks prior to use. The eels were not fed during the experimental period. Exposure at 10 and 100 µL oil/kg fish was carried out, using crude oil obtained from Norman Wells, N.W.T. Calculated amounts of crude oil with a ^{14}C-naphthalene marker were administered by gavage. The amount of labelled naphthalene used was minimal so that it would not alter the naphthalene composition of the crude oil. The oil was mixed with beef-liver homogenate and a total volume of 0.1 mL/dose was given. No regurgitation of the oil occurred, and no gut damage was observed. Experimental animals were given single daily doses containing 0.5 µCi (in both 10 and 100 µL/kg fish) of ^{14}C-naphthalene for 5 consecutive days. Five fish were sampled at each time period of 1,3 and 5 days after initiation of exposure. At the end of the 5 days of dosing, the remaining fish were transferred to another tank to follow the clearance of the oil dose. Again, groups of 5 fish were sampled on days 3, 6 and 12 of clearance. Control groups of fish, which had been force-fed with beef-liver homogenate only, were sampled simultaneously throughout the experimental period and were used to establish analytical and physiological baselines for oil/exposure-related experiments.

At each sampling, fish were anaesthetized in 2% MS222 solution for approximately 3 min. The fish were decapitated and samples of tissues and body fluids were taken. Tissues examined were liver, blood, gall bladder, heart, gills, white muscles, posterior kidney and brain. Extreme care was taken not to puncture the gall bladder during removal and bile samples were taken. In addition, prior to excision, the liver was flushed with a perfusion fluid for fresh water eels (SHUTTLEWORTH 1972) to remove blood trapped in the hepatic circulation. All samples except bile were homogenized, and then extracted with a 1:1 methanol-benzene solution (homogenate:solvent, 1:4). Extraction was carried out for 24 h in teflon-stoppered test tubes on a rotator at 4°C. The mixture was then centrifuged at 1400 xg for 10 min. Aliquots of the organic and aqueous phases were tested separately for ^{14}C-activity. Radioactivity in the solvent phase was used to calculate concentration of crude oil derived fractions while the aqueous layer was used to estimate values for total metabolites. The extraction procedure for the ^{14}C-napthalene oil and its metabolites was found to approach 100% efficiency since re-extraction and total radioactivity counts of the pellet

showed no tracer activity remaining after the first extraction procedure. Further, a test of recovery of known amounts of ^{14}C-naphthalene following the two-phase partition technique was carried out using labelled crude oil. The extraction procedure resulted in an organic/aqueous phase partition coefficient of zero, as no radioactivity above background levels was detected in the aqueous phase. A similar partition coefficient of the ^{14}C-naphthalene was found in bile and liver samples fortified with known quantities of the labelled oil. Aliquots of the organic and aqueous layers were counted by liquid scintillation procedures.

RESULTS

Radioactivity levels in tissues and bile of the American eel, A. rostrata, during and following ingestion of a ^{14}C-naphthalene crude oil at a dose of 10 µL/kg are shown in Fig. 1. Statistical differences among and within treatments were established using a two-factor analysis of variance (significant at $P < 0.05$).

Two different patterns in uptake and release were observed for the solvent extractable fraction. Tissues such as liver, heart, blood, muscle, kidney, and gill showed a significant rise on the third day followed by a drop on day 5 to day 1 levels. No further chanbes in radioactivity were observed between days 5 and 17 in these tissues. A second pattern, characterized by a later peak at the end of the dosing period (day 5), was observed for brain. This increase in radioactivity levels was followed by a gradual drop by day 8 during the depuration period, resulting in tracer levels similar to those of day 1. Radioactivity levels remained unchanged throughout the rest of the experimental period.

The water-soluble fraction, which represents metabolized hydrocarbons, also showed two different patterns in uptake and clearance. With the exception of liver and blood, all tissues examined showed a significant increase in radioactivity at day 5 followed by a drop to day 1 levels during the depuration period at day 8. Bile levels also peaked at day 5. The rate of clearance, however, appeared to be slower than that of the tissues examined because maintained high levels are still observed on day 8. Similarly to the organic solvent fraction results, liver and blood aqueous phases showed a significant increase in radioactivity during the dosing period at day 3. A delay of six days in clearance, however, was observed in the water-soluble fraction of these two tissues when compared to the organic solvent fraction; levels did not drop significantly until day 11.

Total radioactivity was calculated for bile and selected tissues. It represents the sum of tracer levels observed for the aqueous and organic solvent fractions. At all times, the total radioactivity levels in bile were significantly higher than in any other tissue examined. It showed approximately 45 times higher levels of maximum uptake than those observed for the liver which was the tissue exhibiting the highest tracer activity levels.

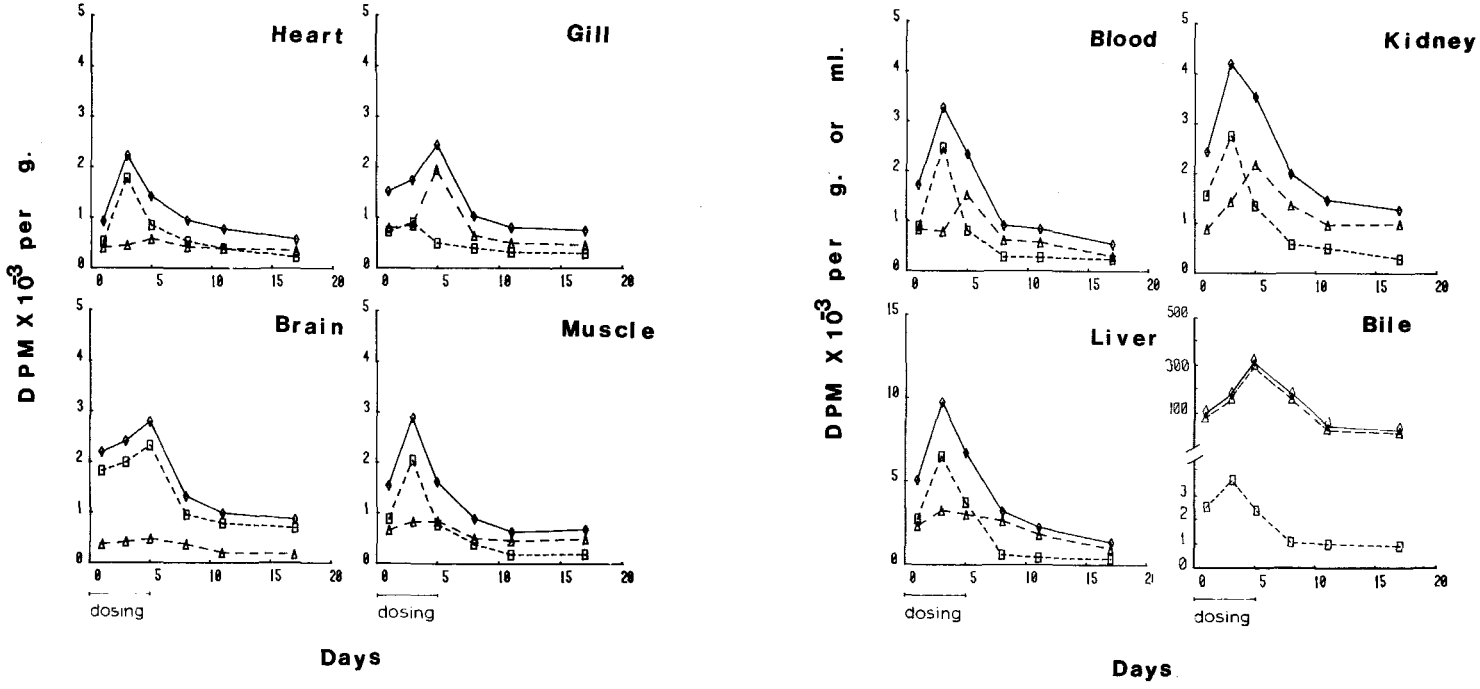

Figure 1. Radioactivity levels in tissues and bile of the American eel, Anguilla rostrata, during and following ingestion of a ^{14}C-naphthalene labelled crude oil at a dose of 10 ppm, given for five consecutive days, 0.05 µCi/dose. (◊———◊, total; □--------□, organic solvent fraction; Δ— — —Δ, aqueous fraction).

The radioactivity levels in selected tissues and bile of A. rostrata during and following ingestion of a 100 µL/kg dose of ^{14}C-naphthalene labelled crude oil were also examined in detail. The patterns of uptake and release were the same as those previously described for the 10 µL/kg oil dose experiment. In addition, no significant absolute differences in the tissue uptake of the labelled naphthalene were found between the 10 and 100 µL/kg exposed fish. It should be repeated that the amount of activity given in both the 10 and 100 µL/kg doses was the same. This suggests a complete absorption of the label from the two dosing regimes. Therefore, as an approximation and based on specific activity of the original dose, the total hypothetical hydrocarbon and metabolite content of the tissues could be estimated. These nominal concentrations of petroleum hydrocarbons are presented in Table 1. It can be seen that there is a ten-fold greater content of hydrocarbons in bile and tissues after a 100 µL/kg dose compared to a 10 µL/kg exposure.

TABLE 1. Nominal concentration[a] in µg/g of petroleum hydrocarbons and metabolites in the American eel, A. rostrata, tissues and bile.

	Concentration (µg/g tissue weight)			
	At peak[c]		At 17 days	
Sample[b]	10 µL/kg	100 µL/kg	10 µL/kg	100 µL/kg
Bile	2,700	28,000	180	1,200
Liver	88	1,000	12	130
Kidney	32	320	12	140
Blood	30	320	5	53
Heart	20	150	5	44
Gill	22	210	7	73
Muscle	26	250	6	76
Brain	25	290	8	74

a = calculated on the basis of ^{14}C-naphthalene specific activity in crude oil doses
b = sample size of 5
c = maximum concentrations at 3 days, except at 5 days in bile, gills and brain

DISCUSSION

The rapid uptake and release of hydrocarbons by the American eel, A. rostrata, was similar to the results obtained with other marine and fresh water fish (LEE et al. 1972, MELANCON & LECH 1978, ROUBAL et al. 1978). The findings indicate that ingestion of petroleum hydrocarbons results in absorption through the gut and deposition in key organs and tissues of treated fish. Tracer activity was mainly concentrated in gall bladder and liver, which are organs associated with the metabolic breakdown and excretion of petroleum hydrocarbons (KORN et al. 1976). The exceptionally high hydrocarbon activity in the bile appears to be mainly due to metabolites since the activity was primarily found in the water-fraction of extracted samples. This is consistent with other studies; for instance, ROUBAL et al. (1977) indicated that intraperitoneal administration of ^{14}C-naphthalene to Coho salmon resulted in maximal ^{14}C-activity in the bile. Further, 70% of the radioactivity was due to the presence of naphthalene metabolites, similar to the findings in the eel. Chromatographic analysis of hydrocarbons in the bile of rainbow trout exposed to naphthalene and methylnaphthalene indicated that at least 65 to 70% of the activity recovered was due to metabolites (MELANCON & LECH 1978). The simple two phase partition technique used to extract bile and tissue samples gives some ready indication on the levels of parent and metabolized hydrocarbons, and might be utilized effectively in other studies of this nature. A ^{14}C-labelled naphthalene was used to study the uptake and discharge of hydrocarbons in the eel because it was considered to be a good representative of the hydrocarbon mixture present in the crude oil. In addition, polycyclic aromatic hydrocarbons such as naphthalene are known to account for most of the toxicity attributed to oil pollution (BLUMER 1971) and have been found to be very potent inducers of hepatic mixed function oxidases in fish (BEND et al. 1974, PHILPOT et al. 1976). The existence of a detoxification mechanism is suggested by the high concentration of parent hydrocarbon and particularly metabolites found in the bile, as well as in the liver, of A. rostrata following oil ingestion. The liver was also found to be a major site of aryl hydrocarbon hydroxylation in the eel (NAVA & ENGELHARDT, to be published), as well as in the other species (MALINS 1977 et al. 1979).

Substantial amount of radioactivity was also found in brain and muscle. Tracer activity, however, was associated mainly with the solvent fraction and radioactivity levels in the water-fraction were almost negligible. The low accumulation of hydrocarbon metabolites is consistent with other reports, indicating relatively low levels of petroleum bio-conversion products in brain and muscle (ROUBAL et al. 1977, MALINS et al. 1979).

Relatively high radioactivity levels associated with the water soluble fraction were also found in other tissues such as kidney, gill and heart. It is not probable that these levels of metabolites are greatly associated with the capacity of these tissues to metabolize petroleum hydrocarbons. A separate study of tissue distribution of aryl hydrocarbon hydroxylase in A. rostrata indicated that the capacity of gill, kidney and heart to metabolize aromatic hydrocarbons is minimal. Presumably then, this water-fraction radioactivity in these tissues was attributable to a passive accumulation, or, especially in the case of kidney and gills, a selective excretion process. Definition of the excretion function in particular will require further study.

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