Fate of Labeled n-Alkanes in the Blue Crab and Stripped Mullet

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A considerable body of knowledge has been accumulated on the distribution and effects of petroleum hydrocarbons on marine life, but relatively little experimental work has dealt with the uptake, discharge, and metabolism of specific hydrocarbon components in higher marine organisms. LEE et al. (1972a) and MORRIS (1973) have shown that several marine invertebrates can rapidly take up and discharge hydrocarbons, with no metabolism of alphatic or aromatic hydrocarbons observed in either the mussel Mytilus edulis or the barnacle Lepas fascicularis. However, another invertebrate, the spider crab Maia squinado, was able to convert napthalene to several water soluble products (CORNER et al., 1973). In contrast to invertebrates, the evidence indicates that fish are able to metabolize aromatic hydrocarbons (LEE et al., 1973b; CORNER, 1975).

Gas chromatographic studies on an oil spill (BLUMER, et al., 1970) showed that the cyclic and aromatic alkanes were very stable over a two month period following the spill, while the n-alkanes disappeared rapidly. BLUMER et al., (1970) stated that this was "presumably a biological modification." While it is well known that many microorganisms can readily metabolize n-alkanes (HAINES and ALEXANDER, 1975, ALBRO, 1970) as can animals (McCARTHY, 1964) and plants (KOLATTUKUDY and WALTON, 1973), the role of higher marine organisms in hydrocarbon metabolism is uncertain.

Because of the limited data on the fate of petroleum hydrocarbons in higher marine organisms, the large amount of hydrocarbon material in the environment to which they are exposed, and the apparent differences in the metabolic capabilities of invertebrates and fish, a study on the uptake, discharge and metabolism of long chain n-alkanes in the blue crab Callinectes sapidus and the stripped mullet Mugil cephalus was undertaken.

Materials and Methods

Blue crabs and stripped mullet were obtained from the Gulf of Mexico and maintained in holding tanks at a salinity of 15 and 12 parts per thousand respectively.

n-Tricosane[R-^{3}H] (4.5mCi/mg) and n-nonadecane[R-^{3}H] (0.5 mCi/mg) were prepared by Wilzbach tritiation, and purified by

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thin layer and column chromatography. Purity was verified by radio-gas-liquid-chromatography (radio-GLC).

Eight liter tanks were filled with water of appropriate salinities, 3 to 6 uCi of n-tricosane(R-^{3}H) added, and the water sonicated. Organisms were then placed in the tanks and removed at the intervals indicated.

In separate studies, labeled n-alkanes were introduced into the digestive tract by dissolving the labeled n-alkane in mazola oil and placing it in the esophagus of fish or by placing the labeled n-alkane on food, and feeding it to crabs which had been deprived of food for 2 days. In all cases, organisms that did not appear to take in the labeled substrate were discarded.

The organisms were sacrificed by freezing. Upon thawing, each organism was disected and specific organs extracted by the method of BLIGH and DYER (1959). Portions of the lipid samples were counted for total radioactivity, and portions were separated into hydrocarbon and more polar lipids by column chromatography on mini-columns of Bio Sil A in Pasteur pipettes. Hexane was used to elute hydrocarbon and chloroform/methanol (2/1) to elute the more polar lipid material. More polar lipid material as used here refers to all lipids more polar than hydrocarbon. Samples were transferred to scintillation vials, solvent removed under nitrogen, 10 ml of scintillation cocktail (0.4% PPO in toluene) added and each sample counted for 10 minutes at 60 percent efficiency.

Some of the more polar lipid fractions were saponified by refluxing in 20% KOH in methanol for three hours, cooled, acidified, extracted with hexane, and methylated according to the method of SCHLENK and GELLERMAN (1960).

Radio-GLC of the labeled substrates and methylated fatty acids was carried out on a 6 ft. x 1/8 in. 3% SE-30 column programmed from 150 to 280°C at 8° per min. A 9:1 stream splitter diverted nine-tenths of the sample through a combustion-flow-through proportional counter.

Results and Discussion

The blue crab was readily able to take up and discharge labeled n-alkane (Fig. 1). After exposure to labeled n-tricosane for 24 hours, an average of 52,000 cpm of labeled hydrocarbon per organism was taken up. However, little of the labeled n-alkane was retained, with an average of 14,000 cpm remaining per organism after 3 days, 9,000 cpm after 7 days, and 4,000 cpm after 14 days. This decrease in n-alkane retained occurred even though the crabs were kept in water containing the labeled n-alkane. It appears that much of the labeled hydrocarbon left the water column and adhered to the walls of the container and particulate matter (BOEHM and QUINN, 1973). Because there was

some labeled alkane in the water, the discharge of n-alkane may be even more complete than indicated in Fig. 1, as labeled material in the crab at 14 days could be material recently taken up and in equilibrium between water and the crab.

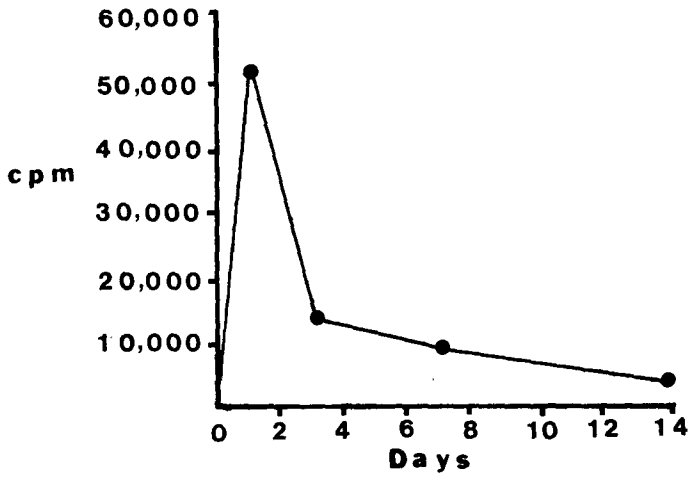

Figure 1. Uptake and discharge of labeled n-tricosane by the blue crab.

The rapid discharge of labeled n-alkane can be partially explained by its location in the crab. Ninety-five percent of the n-alkane taken up at 24 hours was in the gill area, with 70 percent remaining there after 14 days. The only organ to show a consistent increase in labeled n-alkane was muscle, which increased from 2 percent of the label taken up at day one to 10 percent at day 14.

When labeled n-nonadecane was administered by inclusion in the diet, there was a shift in the distribution of the label from the stomach and intestines to the hepatopancreas (Fig. 2). At day 5, 71 percent of the label recovered was in the digestive tract and only 12 percent in the hepatopancreas, while by day 15, the distribution was reversed, with 61 percent of the label in the hepatopancreas and only 12 percent still in the intestine.

Regardless of whether the labeled alkane reached the organism via uptake from water or in food, it was not metabolized by the blue crab. Less than 2 percent of the recovered label was found in the polar lipids, with the rest remaining as hydrocarbon. The distribution of label between n-alkanes and more polar lipids in specific organs was determined during each time period in both routes of administration, with similar results obtained showing essentially no conversion of n-alkanes to more polar lipids. This lack of metabolism of hydrocarbon is consistent with the data reported by others (MORRIS, 1973; LEE et al., 1972a) on other marine invertebrates.

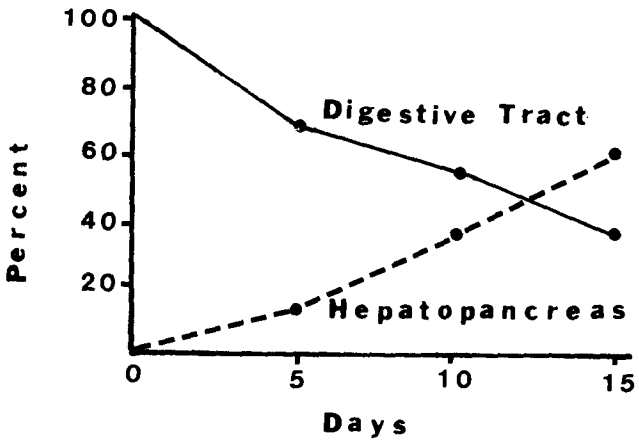

Figure 2. Distribution of labeled n-nonacosane fed to the blue crab. Solid line is percent of recovered label in the digestive tract and the dashed line is percent of recovered label in the hepatopancreas.

In contrast to data from the blue crab, results showed that mullet readily metabolized n-alkanes. Forty-one percent of the n-tricosane-[R-^{3}H] administered into the esophagus was recovered in the more polar lipid fraction at 24 hours. No attempt was made in this study to distinguish between metabolism by microorganisms in the gut or by the fish itself. However, 24 percent of the label remaining in the intestine and stomach after 24 hours was in the form of oxygenated derivatives, whereas 72, 59, and 60 percent of the label recovered in the gills, liver, and heart, respectively, were in the form of metabolic products. This suggests metabolism of the n-alkanes by microorganisms in the gut with selective uptake of the oxygenated derivatives into specific tissues. In accord with this, radio-GLC of the methylated fatty acids derived from n-tricosane[R-^{3}H] indicated that most of the radioactivity was in fatty acids of 17, 19, 21, and 23 carbons, products of the major metabolic pathway for n-alkanes in microorganisms (ALBRO, 1970).

When mullet were exposed to n-tricosane[R-^{3}H] dissolved in water, only 5 percent of the radioactivity taken up after 48 hours was found in the more polar lipid fraction, with 95 percent remaining as hydrocarbon. In this portion of the study, most of the label was recovered in the gills and liver (Table 1). Only 3 percent of the total label recovered was in the intestine, but of this, 17 percent was in the form of more polar lipids (Table 1). The limited metabolism of n-alkanes taken up from the water along with the data showing that the intestine contained the highest percent of metabolic products further suggests that microorganisms in the gut may be responsible for much of the n-alkane metabolism observed in the mullet.

TABLE 1.

Distribution and metabolism of n-tricosane[R-^{3}H] taken up by mullet in forty-eight hours.

Tissue	Percent of recovered label in each tissue	Percent Distribution of label in Hydrocarbon[a]	Percent Distribution of label in More Polar Lipids[a]
Gills	48± 8	98	2
Liver	38± 3	94	6
Heart	10± 7	88	12
Intestines	3± 2	83	17
Stomach	1± 1	92	8

a. Of the recovered label in the whole organism, 95 percent was in the hydrocarbon and 5 percent in the more polar lipid fraction.

Summary

Results of these studies demonstrate that the blue crab was readily able to take up and discharge labeled n-alkanes, but it was not able to metabolize them. In contrast, it appears that n-alkanes taken up via the digestive tract in mullet are readily metabolized, probably via microorganisms in the gut. More limited metabolism was observed in mullet when n-alkanes were taken up via the gills.

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