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ACCUMULATION AND METABOLISM OF PHENANTHRENE IN NORWAY LOBSTER (NEPHROPS NORVEGICUS)

by

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ABSTRACT

[9-^{14}C] Phenanthrene was administered to Norway lobster, Nephrops norvegicus, intragastricly and the radioactivity in different tissues were measured at intervals using a scintillation counter. The radioactivity was rapidly accumulated in the tissues and the highest concentration was measured 1 day after administration. Most of the radioactivity had disappeared 28 days after the administration.

The metabolites of phenanthrene were isolated from the green gland, gonads and intestine. The analyses of the TMS-derivatives of the metabolites were performed using a computerized gas chromatograph mass spectrometer. A total of six metabolites were isolated and identified. Quantitative studies were carried out using selected ion monitoring. The main metabolite found was 9,10-dihydro-9,10-dihydroxyphenanthrene which constituted 78, 95 and 64% of the total metabolic products in green gland, gonads and intestine, respectively. In green gland 41% of the metabolites were conjugated, in gonads 50% and in intestine 23%.

RESUMÉ

(Accumulation et métabolisme de phenanthréne chez la Langoustine, Nephrops norvegicus). Phenanthréne étiquete avec carbone-14 a été administré au Langoustine intragastricment. Le radioactivité dans les tissues différents ont été mesuré par intervalls en employant un compteur á scintillation, Packard Tri-carb. Carbone-14 a été rapidement accumulé dans les tissues différents et la concentration plus hautes ont été trouvées après 24 heures. Apres 28 jours la plupart du carbone-14 est disparue.

Les métabolites de phenanthréne ont été isolées de la glande verte, les gonads et les intestines 48 heures après l'administration de 20 mg. Utilisant la chromatographie en phase gazeuse sur capillaire de verre et des analyses en spectrometre de masse, nous avons analysé les TMS-dérivatives des métabolites. Une somme total de six métabolites a été isolée et identifiée. Des études quantitative ont été accompliés par fragmentographie de masse. Le métabolite principal trouvé, a été 9,10-dihydro-9,10-dihydroxyphenanthréne, qui constituaient 78, 95 et 64% de tous les produits métaboliques, dans la glande verte, les gonads et les intestines respectivement. Dans la glande verte 41% des produits métaboliques ont été conjugé, dans les gonads 50% et dans les intestines 23%.

INTRODUCTION

The disposition of polycyclic aromatic hydrocarbons (PAH's) in marine organisms has recieved an increasing attention in the last decade and many reports have discussed the fate of these xenobiotics in different species of marine animals. Most of these are comprehensively discussed by Varanasi and Malins (1977). Studies involving accumulation and metabolism of PAH given in diet or intragastric to benthos crustaceans are very few (e.g. Corner et al., 1973 and Lee et al., 1976).

In this experiment Norway lobster (Nephrops norvegicus) was used to represent a bottom living crustacean of commercial importance in Norwegian coastal waters. Palmork et al. (1973) detected high concentrations of PAH in the sediments of Norwegian fiords having industries like aluminium smelters, ferro-silicon-, iron and coke-works, and it is therefore expected that benthos organisms in such areas might accumulate these compounds.

MATERIAL AND METHODS

For the radioactiv experiments Norway lobster (both sexes) of a mean weight of 141±64g (S.D.) and 294±45g were used for the metabolic studies. After one week of acclimation in running seawater (7 l/min, 9.5°C, 35°/00 S, 1250 l tank) each animal was given 7,9 µg (0.5 µCi) of [9-^{14}C] phenanthrene (The Radiochemical Centre, Amersham, England) for the ^{14}C-experiment and 20 mg of phenanthrene for the metabolic experiment. The material were dissolved in dimethylsulfoxid (DMSO) and each animal was given 200 µl orally using a 1 ml syringe with a teflon tube attached (ID. 1,25, OD. 1,8mm). The animals were fed with thawed Meganyctiphanes norvegica before and after the dosing of the radioactivity.

The radioactivity was measured at intervals in hepatopancreas, green glands, gonads, heart, muscle, intestine and stomach after dosing, using standard methods. The stomach, however, was extracted with 15 ml of toluene and one ml of the extract was analysed for radioactivity. Soluene-350 and Dimilume-30 (Packard Instrument Co.) and an internal standard ([^{14}C] toluene) were employed in the scintillation counting.

In the metabolic studies the animals (6) were analysed 2 days after dosing. Quantitative and qualitative studies of TMS-derivatized hydroxylated metabolites of phenanthrene in green gland, gonads and intestine were analysed according to Palmork et al., 1978 and Solbakken et al., 1979.

RESULTS AND DISCUSSION

Accumulation and depuration

Table 1 shows the values of radioactivity (as % of administered dose) present after various times in heart, green gland, hepatopancreas, gonads, muscle, stomach and intestine. The greatest concentrations of radioactivity were recovered from hepatopancreas and muscle. In all tissues, except for the intestine, the highest levels of radioactivity were measured 1 day after dosing and after 28 days only minute amounts of the radioactivity were left in the tissues.

Lee et al. (1976) fed blue crab, Callinectes sapidus, with shrimp containing radiolabelled hydrocarbons (benzopyrene, fluorene, naphthalene, methylnaphtalene, methylcholanthrene, hexadecane, heptadecane and dotriacontane) dissolved in ethanol. They found that the fecal material excreted during the first 2 days was high in hydrocarbon (20-50% of total ingested) and they concluded that a great deal was passed directly through the intestinal tract. The experiment with Norway lobster, however, show a low content of radioactivity in stomach (2.7%) and intestine (0.6%) 1 day after administration. The organs analysed contribute 80% of the total given dose. This indicate that most of [14C]-phenanthrene was absorbed from the intestine. It is possible however, that PAH's dissolved in DMSO will be absorbed at a higher rate than PAH's given in food (shrimps).

Lee et al. (1976) found small amounts of radioactivity in green gland and heart in blue crab, they believe, however, that the specific activity for some of these tissues may be high because of their low weight. In Norway lobster 10 and 39% of the concentration (µg/g tissue) in hepatopancreas was found in heart and green gland, respectively, but the percentage of the total dose in these organs is very small (Table 1).

Table 1. Distribution of radioactivity in some organs of Norway lobster at various times following intragastric administration of [9-^{14}C] phenanthrene (7.9 µg/animal).

	1 day	4 days	7 days	28 days
Heart	0.2* (4, 0.2)**	0.1 (3, 0.02)	0.07 (3, 0.02)	0.01 (4, 0.01)
Green gland	0.9 (4, 0.6)	0.2 (3, 0.04)	0.2 (3, 0.1)	0.01 (4, 0.002)
Hepatopancreas	44.3 (4, 24.0)	29.8 (4, 7.2)	19.2 (3, 3.5)	0.8 (4, 0.4)
Gonads	5.6 (4, 3.7)	0.4 (4, 0.2)	0.2 (3, 0.1)	0.2 (4, 0.2)
Muscle	28.8 (4, 17.2)	6.2 (4, 1.9)	4.3 (3, 1.3)	0.4 (4, 0.2)
Stomach	2.7 (4, 2.5)	1.4 (4, 1.7)	0.8 (4, 0.3)	0.04 (4, 0.05)
Intestine	0.6 (4, 0.4)	1.3 (4, 0.4)	1.1 (3, 0.6)	0.04 (4, 0.02)

*) mean value, % of administered dose found in organ.

**) number of animals, standard deviation of mean.

The muscle, however, contain only 3% of the concentration (µg/g tissue) in hepatopancreas but contain 29% of given dose, as the muscle weight are about 40% of the total weight. The concentration in muscle are very high compared with experiments with fish given phenanthrene. Palmork et al. (1978) and Solbakken, et al. (1980) reported only 4% of the administered dose in the muscle of saithe and Solbakken and Palmork (1979) recovered only 6% in muscle of rainbow trout.

Metabolism

The results from the analyses of phenanthrene metabolites in green gland, gonads and intestine are given in table 2. A total of 6 metabolites has been isolated and identified. In contrast to fish dosed with phenanthrene (Palmork et al. 1978 and Solbakken et al. 1979 and Solbakken and Palmork, 1979) the main metabolite in Norway lobster was 9,10-dihydro-9,10-dihydroxy-phenanthrene and not 1,2-dihydro-1,2-dihydroxyphenanthrene. Sims (1962) and Chaturapit and Holder (1978) found the 9,10-compound to be the main metabolite in mammals. This indicate that species differences in metabolic pathways are involved in metabolism of phenanthrene and not the temperature differences between poikilothermic and homoiothermic animals.

In Norway lobster the main metabolite contribute 78, 95 and 64% of the total hydroxylated metabolites in green gland, gonads and intestine, respectively. Unexpectively a high concentration of unchanged phenanthrene was found in green gland and gonads (34 and 65µg/g). Corner et al. (1973) reported the finding of unchanged naphthalene excreted in the urine of dosed crabs, Maia squinado, (qualitative studies). They consider the possibility that the hydrocarbon was attached to organic material (eg. protein) from which it was released when the urine was extracted with n-pentane. According to Palmork et al. (1978) and Solbakken et al. (1979) the concentration of unchanged phenanthrene in bile and urine of saithe were 5 and 12% of the free metabolites, respectively, and Solbakken and Palmork, 1979 found 2% in the bile of rainbow trout. Since the same extraction procedure has been employed in all three experiments (saithe, rainbow trout and Norway lobster) it is unlikely that all the unchanged

Table 2. Concentrations of metabolites in green gland, gonads and intestine of Norway lobster 48 h after intragastric administration of phenanthrene (20 mg/animal).

Compound	Green gland			Gonads			Intestine		
	Unconjugated µg/g	Total µg/g	% Unconjugated	Unconjugated µg/g	Total µg/g	% Unconjugated	Unconjugated µg/g	Total µg/g	% Unconjugated
1-Hydroxyphenanthrene	0.01	0.01	50	*	*	-	0.02	0.11	14
2-Hydroxyphenanthrene	0.01	0.1	5	*	0.03	0	0.05	0.21	23
3-Hydroxyphenanthrene	*	0.02	0	*	*	-	*	*	-
9-Hydroxyphenanthrene	*	0.09	0	0.01	0.02	40	*	0.01	100
Phenanthrene-1,2-dihydrodiol	0.2	0.8	25	*	*	-	0.69	2.01	34
Phenanthrene-9,10-dihydrodiol	1.6	3.5	46	0.5	1.0	51	0.75	4.16	18

* trace (~0,00µg/g)

phenanthrene found in Norway lobster was due to degradation of conjugated metabolites by the extraction procedure and that such high concentration of phenanthrene was attached to organic material. The fraction of free metabolites was 41% in green gland, 50% in gonads and 23% in intestine.

This experiment suggest that Norway lobster should not retain polycyclic aromatic hydrocarbons (PAH) in a PAH polluted area, and this high rate of depuration would diminish the effects of these xenobiotics.

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