environmental dilution on net amino acid efflux may be due to lowered concentrations of Na^+ in the extracellular fluid. A decrease in amino acid concentration of 35% was seen following incubation of brain slices in the ouabain medium. It seems possible that net amino acid efflux may exhibit some sodium pump dependency.

In the elasmobranch model used in this study, amino acids leave the brain rapidly in response to a rapid decrease in plasma osmolarity and at least on this basis do not appear to be solid candidates for the unidentified intracellular osmolytes retained by mammalian brain during rapid hemodialysis. However, even though the skate brain is able to rapidly modulate its intracellular amino acid concentration, species differences in the blood-brain barrier may cloud any conclusion with respect to the mammalian dialysis disequilibrium syndrome.

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DISTRIBUTION OF 1-^{14}C-HEXADECANE IN THE LOBSTER, *Homarus americanus*, AT VARIOUS TIMES AFTER INJECTION INTO THE PERICARDIAL SINUS

Gary L. Foureman, Peter Hall, and John R. Bend, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

The United States is highly dependent upon foreign sources of petroleum for energy production. The increasing demand for these hydrocarbons has resulted in drilling for oil on the outer continental shelf and the transportation of large quantities of crude and refined oil in coastal waters. These developments threaten local marine and estuarine ecosystems in the event of catastrophic spills. The effect of chronic exposure of marine animals to low levels of hydrocarbon pollutants should not be totally discounted, particularly in relation to human health. Many marine invertebrates accumulate lipophilic compounds, such as the carcinogenic polycyclic aromatic hydrocarbon benzo[a]pyrene (Dunn and Stich, Bull. Envir. Cont. Toxicol., 15:398, 1976). Significant quantities of foreign organic chemicals can thus be introduced into our food via this mechanism.

In this report we describe the distribution of hexadecane, a relatively nonvolatile alkane crude oil constituent, in lobster following intracardial administration. The chemical nature of the radioactivity remaining in the hepatopancreas 7 days after dosing was also determined.

Male and female hard-shelled lobsters (360-490 g) were purchased locally (Thurstons, Bernard, Me.). 1-^{14}C-Hexadecane (54 mCi/mmole) was obtained from the Radiochemical Center, Amersham, England. It was diluted with hexadecane (Aldrich Chemical Co.) prior to use and was administered by injection into the pericardial sinus dissolved in Emulphor:ethanol:water (3:3:4, by volume). The lobsters received a dose of 10 mg (4 µCi) ^{14}C-hexadecane/kg. After treatment lobsters were placed in 40 liter aquaria equipped with flowing sea water (12-15°C). The lobsters were fed pieces of herring during the experiment.

Plasma was removed from the pericardial sinus just before sacrifice. Subsequently, the tissues were dissected from the carcass and weighed. Triplicate aliquots of plasma (0.1 ml) and duplicate tissue samples (80-200 mg) were solubilized by digestion in 2 ml NCS (Amersham-Searle) by incubation at 50-60° overnight. Scintillation solvent 18 ml (5 g PPO and 0.25 g POPOP per liter toluene) was added and the radioactivity determined in a Packard Tri-Carb counter. Counting efficiency was determined using the external standard technique and varied from 50-90% for almost all samples.

TABLE 1

SPECIFIC ACTIVITY OF VARIOUS LOBSTER TISSUES FOLLOWING PERICARDIAL INJECTION OF ^{14}C-HEXADECANE (10 mg/kg)

	dpm/mg tissue or /µl plasma TIME AFTER ADMINISTRATION				
TISSUE	24 HOURS	48 HOURS	5 DAYS	7 DAYS	13 DAYS
Hepatopancreas	58.7 ± 9.0 (3)*	51.1 - 62.1 (2)**	63.0 - 71.0 (2)	66.2 ± 5.1 (3)	47.0 ± 15.3 (3)
Green Gland	25.9 ± 3.1 (3)	31.0 - 32.0 (2)	26.9 - 42.9 (2)	45.5 ± 6.5 (3)	27.4 ± 8.9 (3)
Intestine	15.1 ± 3.8 (3)	13.2 - 16.7 (2)	22.0 - 23.4 (2)	29.2 ± 3.7 (3)	17.8 ± 7.2 (3)
Heart	12.9 ± 2.8 (3)	11.4 - 12.3 (2)	13.9 - 14.0 (2)	14.4 ± 3.0 (3)	13.2 ± 2.7 (3)
Egg Masses	7.7 ± 1.2 (3)	6.2 - 9.2 (2)	10.4 - 15.7 (2)	11.7	17.0 - 33.4 (2)
Gill	7.4 ± 1.5 (3)	7.9 - 10.1 (2)	9.5 - 10.9 (2)	10.5 ± 1.9 (3)	7.5 ± 1.2 (3)
Stomach	6.7 ± 2.0 (3)	6.0 - 8.2 (2)	10.0 - 11.7 (2)	17.0 ± 7.3 (3)	8.6 ± 1.5 (3)
Tail Muscle	3.7 ± 0.6 (3)	4.1 - 4.7 (2)	6.4 - 6.8 (2)	5.0 ± 0.3 (3)	6.5 ± 2.6 (3)
Fecal Contents	2.1 ± 1.8 (3)	4.2 - 8.2 (2)	3.2 - 6.8 (2)	6.8 ± 0.4 (3)	23.7 ± 17.2 (3)
Plasma	1.9 ± 0.4 (3)	1.7 - 2.3 (2)	4.2 - 4.5 (2)	3.0 ± 0.9 (3)	2.6 - 3.3 (2)
Male Gonad	--	--	--	21.7 - 23.2 (2)	19.5

*Mean ± SD (N).

**Range, N = 2.

The specific radioactive content of plasma, fecal material and several tissues at various time points after hexadecane administration is shown in Table 1. The hepatopancreas contained higher concentrations of radioactivity (per mg tissue) than any other organ sampled. The hepatopancreas also accounted for 66-73% of the total recovered radioactivity in the lobsters at all time points analyzed (24 hours - 13 days). By comparison, the green gland contained less than 1.5% of the total hexadecane-associated radioactivity although the specific activity was about one-half that of hepatopancreas. Hexadecane was very persistent in all tissues examined including the plasma. There was an increase in the radioactive content of the fecal material (isolated from the intestine) as time progressed. There was no apparent decrease in the specific radioactivity of several tissues over the course of the experiment (heart, egg masses and tail muscle) and $t_{0.5}$ values could not be calculated for any organ due to the persistence of the radioactivity.

The chemical nature of the radioactivity in the hepatopancreas 7 days after ^{14}C-hexadecane administration was examined by thin-layer chromatographic (TLC) analysis on silica gel GF plates (250 µ; Analtech, Inc.) in benzene (solvent A) and benzene:ethyl acetate, 10:1 by volume (solvent B). Repeated ethyl acetate extraction of hepatopancreas homogenate (water, 2 ml plus hepatopancreas, 1 g) removed 96% of the radioactivity. The ethyl acetate extracts were dried and the solvent removed. Duplicate aliquots of the residue were partitioned in hexane: 90% v/v methanol in water. The hexane fraction contained 87% of the radioactivity in each instance, which was virtually all unaltered hexadecane. The aqueous methanol fraction (13% of the initial hepatopancreas radioactivity) contained at least two metabolites, one which co-chromatographed with palmitic acid in solvent systems A and B. Another major radioactive peak migrated on TLC plates (developed in solvent A or B) identically to hexadecan-1-ol and/or hexadecan-2-ol. Whether or not both of these metabolites are present is currently unknown.

The feces (Table 1) appear to be a major site for the excretion of hexadecane and its metabolites. It is interesting that an alkane is so persistent in the lobster following intracardial injection. However, this observation is consistent with the negligible in vitro microsomal mixed-function oxidase activity present in lobster hepatopancreas (Pohl, Bend, Guarino, and Fouts, Drug Metab. Disp., 2:545, 1974). The presence of oxidative metabolites in the hepatopancreas demonstrates that hexadecane is biotransformed in the lobster, although at a very slow rate.

This study has shown that lobster hepatopancreas is an important storage site for hexadecane (and presumably other nonvolatile hydrocarbon isomers). It is well known that this species accumulates other lipophilic compounds into the hepatopancreas (Bend, Hart, Guarino, Rall, and Fouts, Natl. Conf. Polychlorinated Biphenyls, 292, 1976). Consequently, it appears that the lobster (and especially the hepatopancreas) would be an excellent tissue source for sampling hydrocarbon profiles in the marine environment, especially since this species serves as a food source for humans.

SODIUM DEPENDENT TRANSPORT OF β-ALANINE BY ERYTHROCYTES FROM SKATES (*R. eriacea*) ACCLIMATED TO NORMAL AND DILUTE SEAWATER

Leon Goldstein, Joan M. Boylan and Bruce W. Sherman, Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912

We have previously shown that skate tissues contain high intracellular concentrations of free amino acids which play an important role in cell volume regulation. For example, the concentration β-alanine (BALA) in skate erythrocytes is approximately 50 mM. When the fish are adapted to 50 percent seawater (½ SW) BALA concentration falls to 23 mM, reducing the osmotic gradient between the cells and the hypoosmotic extracellular fluid. The mechanism(s) bringing about this reduction in free BALA concentration is the subject of this investigation. Since we found that BALA transport (influx) in skate RBC is Na^+ dependent and that the Na^+ concentration of skate plasma is reduced during environmental dilution, we examined the effects of changes in Na^+ concentration, within the physiological range, on the rates of both influx and outflux of BALA in skate RBC incubated in vitro. In addition we assessed the effects of both rapid and slow acclimation to ½ SW on the rate of influx of BALA into RBC, as measured in vitro.

BALA influx into washed, skate RBC was measured by incubating the cells in a physiological saline medium containing 300 mM NaCl, 5.2 mM KCl, 2.7 mM $MgSO_4$, 5.0 mM $CaCl_2$, 5.0 mM glucose, 0.1 mM BALA (0.25 μCi), 15 mM TrisCl (pH 7.5), 370 mM -urea, osmolarity; 970 mOsm. The cells were incubated for 1 hour at 15 ± 1°C, isolated by centrifugation, washed, extracted with trichloroacetic acid solution and assayed for ^{14}C by liquid scintillation counting. Separate experiments showed that BALA was not metabolized, to any detectable degree, by skate RBC. In the experiments on outflux, RBC were loaded with ^{14}C-BALA by preincubating them with the radioactive amino acid for three hours. The loaded cells were isolated, washed two times with saline solutions containing unlabeled BALA and suspended in physiological saline solution. The cells were then incubated for one hour in physiological saline solution containing nonradioactive 0.1 mM BALA. After incubation the medium was separated from the cells by centrifugation and both the medium and cells were analyzed for ^{14}C by liquid scintillation counting.