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

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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.

Table 1

Effects of medium sodium concentration on rates of influx and outflux of β-alanine by skate erythrocytes incubated in vitro

	300 mM NaCl + 0 mM LiCl	200 mM NaCl + 100 mM LiCl	0 mM NaCl + 300 mM LiCl
Influx β-alanine (μmoles/gRBCxhx10)	1.43 ± .24 (6)	0.82 ± .07* (8)	0.002 (2)
Outflux β-alanine (μmoles/gRBCxhx10)	1.45 ± .02 (28)	0.85 ± .015† (29)	0.65 ± .02† (8)

Values are means or means ± S.E. with the number of assays shown in parentheses. *Significantly different from cells incubated in 300 mM NaCl + 0mMLiCl (P<.05 †<.01)

Table 2

Effects of medium hypoosmolarity on rates of influx and outflux of β-alanine by skate erythrocytes incubated in vitro.

	200 mM NaCl + 100 mM LiCl (970 mOsm)	200 mM NaCl + 0 mM LiCl (780 mOsm)
Influx β-alanine (μmoles/gRBCxhx10)	1.17 ± .11 (9)	1.13 ± .20 (9)
Outflux β-alanine (μmoles/gRBCxhx10)	0.75 ± .03 (8)	1.25 ± 035* (8)

Values are means ± S.E. with number of assays shown in parentheses. RBC were preincubated in elasmobranch saline solution containing 200 mM NaCl + 100 mM LiCl for 2-3 hours before measuring influx or outflux rates. *Significantly different from cells incubated in 200 mM NaCl + 100 mM LiCl.

Table 3

Adaptation of erythrocyte β-alanine transport (influx) during acclimation of skates to dilute sea water (½SW)

Environment	Days	200 mM NaCl + 100 mM LiCl	200 mM NaCl + 0 LiCl
		μmoles β-alanine/gRBCxhx10	
SW	7	0.82 ± .07 (4)	———
½SW	7	———	0.38 ± .04* (5)
	2	———	0.73 ± .21 (5)

Values are means ± S.E. with the number of fish shown in parentheses. Fish acclimated to ½SW for seven days were gradually adapted by reduction of seawater salinity at 10% per day. The other group acclimated to dilute seawater were transferred directly to ½ seawater and kept there for two days. *Significantly different from SW group (P<.01).

As shown in Table 1 lowering the Na^+ concentration in the incubation medium from 300 mEq/L (the concentration of Na^+ in plasma of skates kept in SW) to 200 mEq/L (the plasma Na^+ concentration in skates adapted ½ SW) lowered the rate of BALA influx into RBC incubated in vitro by 43%,

from .143 to 0.082 μmoles/gxhr. A similar effect of medium Na^+ concentration was observed on the rate of BALA outflux from RBC (Table 1); outflux rate dropped from 0.145 μmoles/gxhr in 300 mEq Na^+/L to 0.085 μmoles/gxhr in 200 mEq Na^+/L.

Incubating skate RBC in hypoosmotic (780 mOsm) saline medium (Table 2) had no significant effect on the rate of BALA influx, but BALA outflux was increased 67%; 0.075 μmoles/gxhr in saline medium containing 200 mM NaCl + 100 mM LiCl compared to 0.125 μmoles/gxhr in medium containing 200 mM NaCl + no LiCl.

The effects of short term (2 days) and long term (7 days) adaptation of skates to ½ SW on BALA influx into RBC is shown in Table 3. Short-term adaptation had no effect on the rate of BALA influx. However, gradual adaptation (10%/day for 5 days) to ½ SW reduced BALA influx to nearly 50 percent; the rate of BALA influx was .082 μmoles/gxhr in cells from control (SW) skates incubated in medium containing 200 mM NaCl + 100 mM LiCl and 0.038 μmoles/gxhr in cells from skates adapted to ½ SW and incubated in medium containing 200 mM NaCl with no LiCl.

The BALA concentration of RBC in skates kept in SW is approximately 50 μmoles/g, while in RBC in skates adapted to ½ SW for 7 days the BALA concentration is 23 μmoles/g (Boyd et al., J. Exp. Zool., in press). The present study has shown that extracellular Na^+ concentration and osmolarity as well as acclimation to ½ SW can all affect the rates of BALA influx and outflux in skate RBC. The effect of physiological change in external Na^+ concentration on BALA influx and outflux are of simialr magnitude and, therefore, cancel each other. In contrast lowering medium osmolarity to that found in the extracellular fluid (ECF) of skates adapted to ½ SW increases the rate of BALA outflux by 0.05 μmoles/gxhr. This increased rate of outflux, in the absence of an effect of medium osmolarity on the rate of BALA influx, would lower the BALA concentration in RBC. Assuming that lowering medium osmolarity causes an elevation in BALA outflux by increasing the passive leak of the amino acid from the cells, then the BALA concentration inside the cells would fall in a first order fashion ($k = .001\ hr^{-1}$) to a value of 43 μmoles/g in seven days. This concentration is significantly above the level found in RBC of skates adapted to ½ SW for 7 days. However, the adaptive decrease in BALA influx in skates acclimated to ½ SW will also tend to lower the RBC BALA concentration. The magnitude of this effect is difficult to estimate since the time course of the adaptation in BALA influx is not known. Thus, it is not known whether the effects of ECF osmolarity on BALA outflux and the adaptive decrease in BALA influx can account for the total drop in BALA concentration seen in the RBC of skates acclimated to ½ SW or whether other effects (e.g., adaptive increase in BALA influx) come into play as well.

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THE BLOOD-BRAIN BARRIER OF THE SPINY DOGFISH, *Squalus acanthias*; APPLICATION AND CORRELATION WITH PUBLISHED ANTINEOPLASTIC DRUG TESTING IN A MOUSE BRAIN TUMOR SYSTEM

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The treatment of brain, spinal cord, and meningeal tumors by the intravascular administration of antineoplastic drugs has met with only limited success. Since the capillaries of the central nervous system (CNS) are relatively impermeable to polar materials (the so-called blood-brain barrier phenomena), the ineffectiveness of many of the drugs which have been tried may be a result of the slow or insignificant exchange of the drug from blood to CNS and cerebrospinal fluid (CSF).