was either completely omitted or substituted stepwise by choline chloride. At half control levels of Na taurine uptake dropped 40%, from .092  $\mu$ moles/g wet wt per hr to .054. The drop was linear down to an approximate 80% drop in taurine uptake (.017+.004  $\mu$ moles/g per hr; SEM = .003) with complete replacement of Na in the medium. Thus Na dependence in skate atrium is in agreement with similar findings in mammalian retina, platelets, kidney, heart slices, fetal heart, and brain which have also demonstrated that Na was required for maximal rates of taurine transport (Grosso, D.S. et al., J. Clin. Invest. 61: 944-952, 1978).

The effect of an osmotically diluted medium on the uptake rates of [14C] taurine by skate atrium was tested, first, by diluting both NaCl and urea one-third to achieve an osmolality of 700 mosmoles/1 and, second, by keeping the NaCl at the usual level of the control medium (280 mM) while achieving the same diluted medium observed in the plasma of environmentally diluted skates (50% sea water) by correspondingly reducing only the urea content of the in vitro medium. In internal paired controls taurine uptake in the former was reduced by 78% of control uptake and only by 46% in the dilution experiment containing "normal" Na levels. The decreases are significantly different from each other with a probability P value of < .05 according to Student's t distribution. Thus in elasmobranchs such as Raja erinacea, where the osmolality of plasma usually drops 30% as the fish encounter diluted sea water, the osmoregulatory role of amino acids in cell volume regulation may be accounted for by Na dilution, per se, rather than due entirely to some general osmolality effect on intracellular solute content. This would be particularly apt where taurine in critical tissue such as brain or heart constitutes 50% or more of the total free amino acids (Forster, R.P. et al, Comp. Biochem. Physiol. 60A: 25-30, 1978).

Ouabain  $(10^{-4}\text{M})$  decreased control taurine uptake 41.28% (P<.01) corrected for ECS, in contrast to mammalian heart where in identical concentrations it actually increases taurine uptake by more than 25%. Na azide  $(10^{-2}\text{M})$ , an inhibitor of electron transport between cytochrome oxidase and molecular oxygen, decreased taurine uptake 75.23% (P<.001). In confirmation of last summer's work on skate atria which indicated that the taurine transport mechanism is selective for  $\beta$ -amino compounds, the taurine analogue  $\beta$ -alanine (0.5mM) diminished taurine uptake 57%, whereas related  $\gamma$  and  $\alpha$  amino acids such as  $\alpha$ -aminobutyric acid and  $\alpha$ -aminoisobutyric acid had no significant competitive inhibitory effects. Similarly the following compounds previously shown to have a relationship between  $\beta$ -adrenergic stimulation and increased taurine influx in mammalian hearts; isoproterenal, dibutyryl cyclic AMP and theophylline, were ineffective in altering rates of taurine uptake. The skate, as with all elasmobranchs, lacks sympathetic adrenergic cardio-accelerator fibers to the heart (Burnstock, G. Pharm. Rev. 21: 247-324, 1969).

## Conclusions

There are significant redistributions of intra- and extracellular water that result from the use of certain agents and other conditions used to characterize membrane transport systems, in this case the demonstration of a carrier mediated system for taurine in the myocardium of the skate atrium. Taking corrections for ECS content into account our in vitro studies show that taurine was accumulated intracellularly against a steep concentration gradient in atria incubated in 0.1 mM taurine by a system that was temperature dependent, required sodium, strongly inhibited by  $\beta$ -alanine, and unaffected by ouabain and  $\beta$ -adrenergic stimulation. Supported by NIH grant HLO4457.

## FURTHER STUDIES OF THE EFFECTS OF PETROLEUM HYDROCARBONS ON MARINE BIRDS

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Recent laboratory and field studies with Herring gulls (Larus argentatus) and Black Guillemots (Cepphus grylle) have indicated that small oral doses of ingested crude oil cause a number of physiological aberrations in young seabirds,

the most dramatic of which is depressed growth rate (Miller et al, Science 199:315, 1978; Butler and Lukasiewicz, Auk 96:809, 1979; Peakall et al, Ambio, in press, 1980). In the present study we address the following questions: 1) which components of the complex crude oil mixture are responsible for the observed effects, 2) whether the toxic effects of crude are modified by emulsification with a dispersant that might be used at the site of an oil spill and 3) what are the effects of crude oil dosing on young and adult birds of another species.

Herring Gull chicks were collected on Old Man Island, Maine, and maintained at the Laboratory as previously described (Science 199: 315, 1978). Leach's Storm Petrel (Oceanodroma leucorhoa) chicks and adults were maintained in their burrows on Little Duck Island, Maine. Experimentals were given a single oral dose by intubation of either a weathered South Louisiana crude (WSLC), an unweathered Prudoe Bay crude (PBC), an aromatic fraction of PBC, an E.P.A. approved dispersant (Correxit, Cor) or a 10/1 (ail/dispersant emulsion of PBC and COR); controls received corn oil. Gulls were weighed daily; petrel chicks were weighed every 3 d. At the end of the experiment, gulls and adult petrels were sacrificed and the livers, nasal glands and adrenals were weighed.

Previous gull experiments indicated that aromatic, but not aliphatic, compounds in WSLC were responsible for the observed depression in chick growth rate (Miller et al, <u>Bull. M.D.I.B.L.</u> 18:74, 1978). This year we split the aromatic components of PBC into 2 fractions, one (AR-1) containing those compounds with 3 or less rings and another (AR-2) containing those compounds with 4 or more rings. Experimental gull chicks (7-8 birds per group, mean initial body weight 401 ± 29 g) received 1 ml of PBC or the amount of AR-1 or AR-2 equivalent to that found in 1 ml of whole oil. Additional experimental gulls were given 0.1 ml of a commercially available dispersant (COR) or 1 ml of a 10/1 PBC/COR, emulsion. Birds dosed with whole oil or AR-2 exhibited depressed growth rates (Fig. 1) and increased nasal

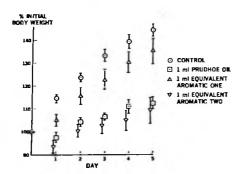


Figure 1. Effect of PBC or two of its aromatic fractions on Herring Gull chick weight gain (expressed as mean  $\pm$  SE). T-test comparisons with control birds indicated that only whole oil and the AR-2 groups exhibited statistically significant depressions in weight gain (p < 0.01).

gland and adrenal weights (Table 1) when compared to controls; AR-2 dosing also caused liver hypertrophy. These results are qualitatively similar to those reported previously for weathered and unweathered SLC (Miller et al, Science 199:315, 1978; Bull. M.D.I.B.L., 18:74, 1978). They indicate that the higher molecular weight aromatic compounds in PBC are responsible for the physiological effects observed after oil ingestion.

The question of dispersant effects on oil toxicity in birds is of importance when decisions have to be made with regard to the least disruptive means of dealing with oil spills. Our study indicates that birds dosed with COR dispersant showed no significant changes in growth rate or organ weights when compared to controls and that those dosed with the PBC/COR emulsion showed no differences from birds dosed with oil alone (growth data not shown, organ weights in Table 1). In contrast, studies with marine invertebrates and teleost fish indicate that certain dispersants are clearly toxic when added to sea water and that these dispersants potentiate the toxicity of oil (Swedmark et al, Water Res. 7:1649, 1973; Butler et al, in preparation).

Table 1. Effect of PBC, PBC aromatic fractions (AR-1, AR-2), dispersant and emulsions on organ weights of Herring Gull chicks

Treatment (n)	Nasal Gland mg/kg	Adrenals mg/kg	Liver g/kg
Control (8)	417 <u>+</u> 23	155 <u>+</u> 15	44.6 + 2.0
1.0 ml oil (7)	654 <u>+</u> 45	217 + 7 *	43.8 + 2.2
1.0 ml equiv. AR-1 (7)	491 <u>+</u> 34	147 <u>+</u> 7	46.7 ± 3.1
1.0 ml equiv. AR-2 (6)	741 <u>+</u> 28 *	267 <u>+</u> 20 *	60.6 + 2.3 *
).1 ml Dispersant (6)	490 <u>+</u> 34	155 <u>+</u> 11	41.0 + 1.1
1.0 ml Emulsion (6)	607 + 24 *	238 + 2 *	41.0 + 2.9

Data expressed as mean + standard error. Birds collected 5 d after dosing. Results of t-test comparisons with controls indicated ( $^{\frac{1}{4}}$  p<0.01).

Our preliminary field studies with petrels indicated that chicks (mean initial body weight 33 ± 2 g, 11-12 birds per group) dosed with 0.05 ml of either PBC, Atlantic/Pacific (AP) dispersant or a 10/1 PBC/AP emulsion exhibited no differences in weight gain or other growth parameters from paired controls (3 week growth data, not shown). On a body weight basis, these chicks received oil doses that were roughly equivalent to those causing significant depression of growth in gulls (above). With adult petrels, 0.1 ml doses of either PBC or WSLC (administered during the broad phase of the reproductive cycle) caused significant adrenal and nasal gland hypertrophy (Table 2). Of potentially

Table 2. Effects of ingested crude oil on adult Leach's Storm Petrel organ weights

Treatment (n)	Nasal Gland mg/kg	Adrenals mg/kg	Liver g/kg
Controls (11)	774 <u>+</u> 22	177 ± 8	25.8 + 0.6
.1 ml WSLC (5)	957 + 77 *	296 + 40 *	25.7 + 0.9
.1 ml PBC (7)	966 + 29 *	215 <u>+</u> 6 *	$25.4 \pm 0.7$

Data expressed as mean + SE. Birds collected 14-21 d after dosing. Results of t-test comparisons with controls indicated  $(\bar{x} p < 0.01)$ .

greater importance, only 53% of the chicks of dosed parents survived, compared with 100% survival for controls. These data suggest that petrel chicks may be considerably less sensitive to ingested oil than gulls or guillemots, and that petrel adults, exposed to ail during the reproductive cycle, are less successful in raising their young. This latter fin in clearly requires further study. Supported by U.S.P.H.S. grant ES 00920.

CHARACTERIZATION OF A STEROID-BINDING PROTEIN IN THE SPINY DOGFISH, <u>SQUALUS ACANTHIAS</u>
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The elasmobranchs are of particular evolutionary importance and great biological interest in their reproductive physiology. Sex steroid hormones have been identified in this group and levels of androgens and estrogens reach con-