

Figure 3. Effect of VIP and theophylline on intracellular cyclic AMP level in rectal gland slices. Cyclic AMP was measured by protein kinase binding assay (Stoff et al., PNAS 69:805-808, 1972). VIP increases intracellular cyclic AMP content at each concentration tested and the addition of theophylline results in a synergistic effect. Secretin has no effect. All values are means  $\pm$  SEM.

studied the effect of somatostatin on the VIP response in the rectal gland (Figure 4). In these studies, VIP ( $10^{-6}$  M) increased both the volume and chloride secretion rate while somatostatin ( $1.4 \times 10^{-7}$  M) alone had no effect, but completely blocked the VIP induced response. In other studies, not shown here we found that this effect of somatostatin was reversible. Furthermore, somatostatin had little or no effect on theophylline or cyclic AMP induced secretory response.

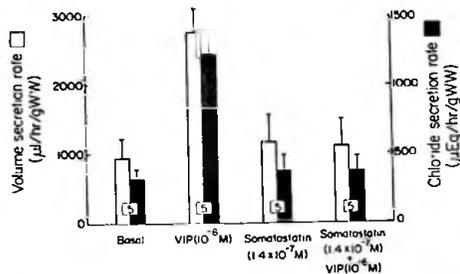


Figure 4. Effect of VIP and somatostatin on volume and chloride secretion rate in the isolated perfused rectal gland. Somatostatin has no effect alone but inhibits the secretory response to VIP. All values are means  $\pm$  SEM.

These studies demonstrate that vasoactive intestinal peptide stimulates active chloride transport by a mechanism mediated by cyclic AMP. This response is prompt, dose related and reversibly blocked by somatostatin. Since somatostatin completely inhibited the VIP-induced secretory response but had little effect on theophylline or cyclic AMP-induced secretion it seems likely that somatostatin exerts its effect by preventing the VIP induced increase in cyclic AMP. Recent studies indicate that both VIP and somatostatin are present in the plasma and gastrointestinal tract of these cartilagenous fish as well as in mammals (Falkmer et al., Metabolism 27 (Suppl. 1):1196, 1978). The precise role of these peptides in the regulation of chloride transport in the rectal gland remains to be established.

#### FIELD STUDIES OF CRUDE OIL TOXICITY IN SEABIRDS

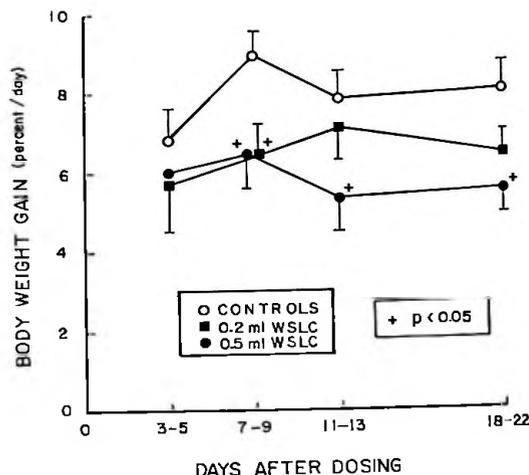
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Recent investigations indicate that sublethal doses of crude oil can result in decreased weight gain, hypertrophy of adrenal, nasal and hepatic tissue, decreased efficiency of intestinal transport, and decreased osmoregulatory ability in young seabirds raised under controlled laboratory conditions (Miller et al., Science 199:315-317, 1978). It is clear that such studies must be extended to wild populations to facilitate our understanding of the biological impact of environmental contamination on marine avifauna. To this end, a field team established a camp on Little Duck Island, Maine, in

the summer of 1978 to test the applicability of previous laboratory results to seabirds living in the wild. We examined the effects of a weathered South Louisiana crude oil (WSLC Miller et al., Bull. MDIBL 17:40-42, 1977) on Herring Gull (*Larus argentatus*) and Black Guillemot (*Cephus grylle*) chicks. Pilot studies were also conducted (1) to determine whether oil dosing influenced selected behavioral parameters in gull chicks and (2) to evaluate Leach's Storm Petrel (*Oceanodroma leucorhoa*) as a suitable test species for assessing the impact of oil and other marine pollutants on free-living, adult seabirds.

Herring Gull chicks (200 - 500 g) were banded at the rest site and fed (via intubation) 1 ml of corn oil, 0.2 ml WSLC + 0.8 ml corn oil, or 0.5 ml WSLC + 0.5 ml corn oil. Growth parameters (i.e., weight gain, culmen length and toe length) were recorded at the time of dosing and at 4- to 8-day intervals; thereafter, mean initial weights for the 3 groups were not statistically different. By day 7 - 9, both treatment groups exhibited significant decreases in rate of weight gain when compared to controls (Figure 1). Birds in 0.2 ml group had recovered by days 11 - 13, but the 0.5 ml group showed reduced weight gain up to 18 - 22 days after dosing. The 0.5 ml group showed a 21% decrease in

Figure 1. Effects of WSLC ingestion on body weight gain in Herring Gull chicks. Rate of weight gain is expressed as percent change per day (mean  $\pm$  SE). Each point represents data from 9 - 22 birds. There were no statistical differences between the initial body weights of the control ( $\bar{x}$  = 341  $\pm$  15 g), 0.2 ml ( $\bar{x}$  = 366  $\pm$  21 g), or 0.5 ml groups ( $\bar{x}$  = 379  $\pm$  19 g).



survivorship to 700 g and/or 20 days following dosing, when compared to the 0.2 ml or control birds. Culmen growth was significantly depressed after 7 - 9 and 11 - 13 days for both treatment groups, but toe growth was unaffected. Growth data for Black Guillemot chicks dosed with WSLC followed the pattern previously reported (Miller et al., Bull. MDIBL 17:40-42, 1977). Treatment birds showed a significant depression in rate of weight gain, and a clear dose response was evident between the 0.2 ml and 0.5 ml groups.

Behavioral observations of experimental and control gull chicks were conducted for 32 days following dosing. Behavioral data were recorded from 07:00 - 12:00 hrs each day utilizing cassette tape recorders and super-8 mm movie cameras with intervalometers. Preliminary analysis of taped data indicate no statistical differences between control and treatment groups for several behavioral parameters including: frequency and duration of food soliciting, foraging, maintenance behavior (e.g., preening, resting, stretching, etc.) and social interaction with parents. Since birds were dosed at 12 + days of age, pollutant induced alterations in behavioral ontogeny could not be thoroughly evaluated.

Adult Leach's Storm Petrels were captured and banded in their burrows, dosed with either WSLC (0.1 ml) or corn oil, and released. Four oil-dosed petrels recaptured 2 weeks later exhibited a significant increase (65%) in adrenal gland weight and a slight increase (18%) in nasal gland weight when compared to 6 recaptured control birds. Chicks of oil-dosed adults also demonstrated a trend

toward decreased rate of weight gain (due possibly to parental neglect or cross-contamination from dosed parents). This pilot study with petrels indicates that this species is well suited for pollutant toxicity research, since petrels are abundant, easily accessible in their burrows, and available for long periods during the incubation and chick phases of their breeding cycle.

The results of this successful field study confirm and extend previous laboratory work and emphasize the significant impact that oil pollution could have on young seabirds in the wild. Future research plans involve the effects of oil on the reproductive behavior and physiology of adult seabirds, the behavioral ontogeny of chicks, and mechanisms of contamination of chicks by adults. The impact of other pollutants such as oil dispersants and organochlorines will also be investigated. This study was supported by United States Public Health Service Grant ES 00920.

#### EXPERIMENTS ON THE CELL VOLUME REGULATION IN SLICES OF THE RECTAL GLAND OF THE DOGFISH (*Squalus acanthias*)

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The mechanism(s) of volume regulation in the cells of the dogfish rectal gland was investigated in the light of a pilot experiment carried out jointly with Dr. J. Hayslett in 1972 showing, that as compared with controls, an inhibition of the  $\text{Na}^+$ -pump by 0.5 mM ouabain produced only a slight swelling of the tissue, suggesting the presence of an ouabain-insensitive component of the regulating system.

Slices cut perpendicular to the long axis of the gland (mean thickness 0.3 - 0.4 mm) were employed. The slices were prepared free-hand by the method of Deutsch (J. Physiol. 87:568, 1936), and the fibrous capsule was trimmed off. Incubation media: Dogfish Ringer salines were employed; modifications thereof will be mentioned below. In a number of experiments the salines contained  $^3\text{H}$ -polyethylene glycol (PEG) (1 mg/ml; 1  $\mu\text{Ci}/\text{ml}$ ) as a marker of the extracellular fluid compartment. Samples of tissue were taken: (a) fresh; (b) after loading the cells with  $\text{Na}^+$  by incubation of the tissue in an isotonic  $\text{Ca}^{2+}$ -free saline at  $0^\circ\text{C}$  in the absence of any metabolizable substrate; (c) after incubation of the loaded tissue slices in salines containing glucose as metabolizable substrate. Usually, 10-12 slices (each weighing approximately 10 mg wet wt) were incubated in 2.5 ml saline under appropriate conditions. All tissue samples were gently blotted, and two slices were combined for one analytical procedure. The blotted slices were weighed, and then dried at  $85^\circ\text{C}$  overnight. Controls showed that all tissue water was lost under these conditions. The differences between wet and dry wt (W.W. and D.W.) of the tissue were taken to represent tissue water. The dry tissue samples were extracted for not less than 48 h with 5 ml 0.2 N  $\text{HNO}_3$ . In this extract, tissue cations ( $\text{Na}^+$  and  $\text{K}^+$ ) were determined by flame photometry,  $\text{Cl}^-$  by potentiometric titration, and the activity corresponding to tissue PEG was assayed by scintillation spectrometry. The means and S.E. for each experimental group were determined. The data are expressed in kg  $\text{H}_2\text{O}$  or mequiv. electrolytes per kg tissue D.W. The extracellular space E is given in kg/kg tissue W.W. From the obtained values, the apparent intracellular ionic concentrations may be computed using the usual simplifying assumptions (e.g., availability of all cell water as solvent, uniform distribution of electrolytes in cell  $\text{H}_2\text{O}$ , no binding or other interaction of electrolytes with cell constituents, etc.).

The determined parameters for fresh tissue, tissue after loading at  $0^\circ\text{C}$ , after aerobic incubation ( $\text{O}_2$ ), and the effect of 0.5 mM ouabain thereon, are given in Table 1. It will be seen that in the course of loading the tissue swells slightly by 0.44 kg  $\text{H}_2\text{O}/\text{kg}$  D.W.  $\pm$  0.08 (S.E.), and this process is associated with a marked uptake of  $\text{Na}^+$  (and  $\text{Cl}^-$ ) and some loss of  $\text{K}^+$ . Subsequent aerobic incubation of the loaded tissue did not produce a net loss of water, although regularly a reaccumulation of  $\text{K}^+$