In a field study (conducted on Little Duck Island), Leach's Petrel (Oceanodroma leucorhoa) burrows were assigned to a control or one of two experimental groups. For one experimental group, one adult bird was given a single 0.1 ml dose of PBC by intubation; for the other, both adults were dosed. Controls were sham dosed. Experimental burrows were further divided into those with adults dosed when their chicks were 3-5 days old and those with adults dosed when their chicks were about 15 days old. Dosing the adults caused a significant decrease in survival among the 3-5 day chicks (16 of 16 controls survived; of the 23 chicks from burrows with 1 dosed adult, 16 survived; and of the 21 chicks from burrows with 2 dosed adults, 11 survived), however, the weight gain of the survivors was little affected. With 15 day chicks, no mortality was found in experimental or control chicks, but weight gain was significantly reduced for 3-6 days after adults were dosed (Fig. 2). Eventually experimental

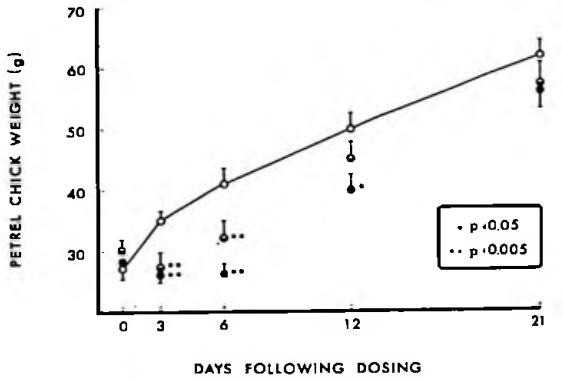


Figure 2.--Weight gains for petrel chicks (15 day old when adults dosed) from burrows in which adults were either sham-dosed (controls) or fed 0.1 ml of PBC. Data given as mean + SE for 8 (control, open circles), 8 (1 adult dosed, half-closed circles) or 9 (2 adults dosed, closed circles) chicks.

chicks appeared to recover; however, even after 21 days, the growth rates (for the entire experimental period) were slightly, but significantly lower than controls. In this experiment, we measured nest visitation by adults and found no differences between any of the groups, indicating that adults were not deserting after they were dosed. We also dosed 15 day old petrel chicks directly and found no reduction in weight gain; taken together, the data suggest that the adult's ability to provide food for their young is impaired by oil dosing. In support of the hypothesis that oil affected adults, we have found that when adults were given 0.1 ml of PBC, released and then recaptured 2 weeks later, nasal and adrenal gland weights and plasma thyroxine levels were increased over controls (Butler et al., Bull. MDIBL, 19: 33, 1979; Peakall et al., op. cit.). These findings point to a previously unsuspected mechanism of oil toxicity in seabirds. Further studies are clearly needed to determine the environmental significance of the observed effects. (Supported by USPAS Grant ES 00920.)

## EFFECT OF INFUSIONS OF SALINE IN THE DOGFISH IN VIVO ON THE RATE OF SECRETION BY THE RECTAL GLAND

R. Solomon, R.M. Rosa, J.S. Stoff, P. Silva and F.H. Epstein, Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory, Beth Israel Hospital, Department of Medicine, Beth Israel Hospital and Harvard Medical School

While considerable advances in our understanding of the physiology of the rectal gland have been made in recent years, a number of important questions remain unanswered. In particular, little is known about the hemodynamic, neural and hormonal agents that control rectal gland secretion. One hormone, vasoactive intestinal peptide, has been identified by Stoff in the plasma of the dogfish shark and has been shown to stimulate rectal

gland secretion in vitro. The pioneer studies of Burger on the intact fish suggested that expansion of the extracellular fluid volume (ECF) or increases in ECF osmolality also stimulated rectal gland secretion. However, these experiments are technically difficult to perform in the unanesthetized fish and are quite variable in results. The purpose of the present investigation was to develop an in vivo model for more precise and quantitative studies of the factors which control rectal gland secretion.

Female - spiny dogfish, Squalus acanthias, weighing between 3 and 5 kg were studied while free swimming or after pithing. The fish were pithed by passing a stiff piano wire through a 1 cm midline incision at the tip of the snout down the entire length of the spinal column. After pithing the fish remained completely immobile, while able to gill spontaneously. The rectal gland duct was catheterized with PE 90 tubing after everting the cloaca (Solomon et al., Bull., MDIBL., 14: 122, 1974). Rectal gland blood flow was determined in some fish by direct measurement of venous flow collected with PE 60 tubing placed in the rectal gland vein through a ventral abdominal incision. These animals were heparinized (600-800 U/fish) and maintained on a shark board with head and gills under water. Blood was returned to the fish at the end of each collection period. Hourly collections of duct fluid and venous effluent were made for 1 to 2 hours before and 3 hours after injection of either isotonic shark Ringers (150 ml) or hypertonic 1 M saline (50 ml).

Both isotonic shark Ringers and hypertonic saline produced impressive stimulation of rectal gland secretion in the pithed fish (Table 1). In the unstimulated state, the mean duct flow rate was 0.08 ± .04 ml/kg/h for 13 fish, a value

Table 1.--Effects of Intravenous Infusions on Rectal Gland Secretion in Squalus acanthias

| Solution (n) | | Duct Flow Rate ml/kg/h | Chloride Concentration mEg/L | Chloride Secretory Rate μEg/kg/h |
|---|---|---|---|---|
| Isotonic (6) Shark Ringers | C | 0.15 ± .09 | 492 ± 27.1 | 75.0 ± 49.1 |
| | E | 3.06 ± .61* | 553 ± 7.6 | 1673 ± 329* |
| Hypertonic (7) Sodium Chloride | C | 0.02 ± .01 | 500 ± 12.6 | 15.9 ± 4.0 |
| | E | 1.61 ± .43* | 545 ± 8.3** | 884 ± 236* |

C-Control period
E-Peak values during experimental periods
*-$P < 0.001$ by paired "t" test
**-$P < 0.05$ by paired "t" test

lower than that found in free swimming fish (0.39 ± .18 ml/kg/h, 10 collections in 5 fish). Following the intravenous infusions of saline, the rate of duct flow and the rate of chloride secretion rose dramatically to peak values 100 to 300 times greater than baseline. The isotonic saline infusion induced a greater increase in rate of flow and chloride secretion than hypertonic infusions although this difference did not reach statistical significance. In addition, the response with isotonic shark Ringers solution was more immediate ($p < 0.05$ at one hour) than fish infused with hypertonic saline (Fig. 1). In five pithed fish, blood flow to the rectal gland averaged 3.31 ml/kg/h, a rate similar to that reported by Kent using microspheres (Kent et al., Bull. MDIBL, 13: 64, 1973). Blood flow increased with infusion of either isotonic (2 fish) or hypertonic (3 fish) solutions and closely paralleled changes in duct flow rate. No differences in peak blood flow were seen between fish receiving either isotonic or hypertonic infusions (Table 2).

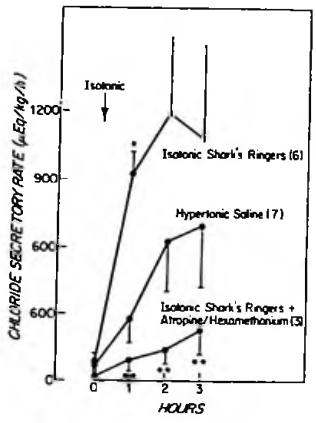


Figure 1.--The time course of rectal gland chloride secretion in response to infusion of either isotonic shark Ringers solution or hypertonic saline. *p<0.01 compared to basal secretory rate. **p<0.01 comparison of secretory rate between isotonic infusion alone and following pretreatment with atropine and hexamethonium.

Table 2.--Effects of Intravenous Infusions on Rectal Gland Blood Flow and Chloride Secretory Rate

| | Blood Flow Rate ml/kg/h | Chloride Secretory Rate μEg/kg/h |
|---|---|---|
| Control | 3.31 ± 0.73 | b |
| Experimental[a] | 9.73 ± 1.76* | 335 ± 121 |

[a]Experimental data represent pooled studies from 2 fish after isotonic expansion and 3 fish after hypertonic infusion.

[b]Obtainable in only 2 fish before stimulation

*p<0.001 by paired "t" test.

Treatment of fish with the combination of atropine (1 mg/kg) and hexamethonium (5 mg/kg) blocked the secretory response to infusion of isotonic shark Ringers solution (Figure 1, n=3). In five additional fish, the stimulatory response to hypertonic saline was inhibited after injection of atropine and hexamethonium. Hemodynamic measurements were not made in any of these studies.

The present study was designed to develop a model to study the factors which modulate rectal gland function in vivo. The pithed animal preparation is ideal in this regard because it permits reproducible collections of even small volumes of secretion from the rectal gland duct while simultaneously measuring hemodynamic parameters. It is also evident that stimulation of rectal gland secretion can be achieved with either isotonic or hypertonic saline expansion (Table 1). Although the response to isotonic infusion is of greater magnitude and more rapid onset when compared to the hypertonic infusion, both the volume and osmolar load infused were greater with the isotonic infusion. These factors may account for the observed differences in response.

While the present studies indicate that an intact central nervous system is not required for the stimulatory effect of saline infusion on rectal gland secretion, the role of the parasympathetic nervous system on rectal gland secretion is less certain. Burger noted that atropine depressed and even blocked rectal gland secretion in free swimming fish (Physiol. Zool. 35:205, 1962). He considered this inhibition non-specific, since local denervation with piperocaine and administration of parasympathomimetic agents did not after gland function. Studies

of the hemodynamic effects of parasympathetic inhibition by Kent et al., documented a 30% decrease in cardiac output with a 12 mg/kg dose of atropine and a smaller decrease following surgical vagotomy (Bull. MDIBL 16:66, 1976). In contrast, Opdyke et al., found only minor decreases in blood pressure with either atropine (0.1 mg/kg) or hexamethonium (10 mg/kg) (Bull. MDIBL 18:32, 1978). In the present studies, a combination of both atropine and hexamethonium at doses intermediate to those employed by Kent and Opdyke were administered. Such dosages appear to inhibit rectal gland secretion in response to saline infusion, but the experiments do not allow distinction between a direct inhibitory effect on gland secretion and an indirect effect secondary to decreased cardiac output and rectal gland flow.

Similarly, the enhanced rectal gland secretion in response to saline infusion may be mediated directly by increased rectal gland blood flow or indirectly by activation of a hormonal or neurogenic stimulus to rectal gland secretion. Though blood pressure does not increase with either 150 ml of isotonic shark Ringers (Kent, et al., Bull. MDIBL. 14:55, 1974) or 30 ml of 1 M NaCl (Opdyke, et al., Bull. MDIBL. 12:73, 1972) in Squalus acanthias, it is not known whether cardiac output is altered by such maneuvers. It seems unlikely, however, that the three-fold increase in rectal gland blood flow can be entirely accounted for by an increase in cardiac output and furthermore that a three-fold increase in rectal gland blood flow can induce a 10-20 fold increase in the rate of secretion of the rectal gland.

## ISOTONIC SOLUTIONS STIMULATE SECRETION BY THE "IN SITU" RECTAL GLAND OF THE DOGFISH

D. Erlij, R. Rubio and P. Silva, Dept. of Physiology, SUNY Downstate Medical Center, Brooklyn, New York; Dept. of Physiology, School of Medicine, University of Virginia, Charlottesville, Va.; Dept. of Medicine, Harvard Medical School, Boston, Mass.

The rate of secretion of the rectal gland of the dogfish varies over a wide range of values. The identification of the hormones or transmitters that control secretion rate requires prior knowledge of the variables in the internal environment of the animal that are capable of provoking secretion. The pioneer studies of Burger (Physiol. Zool. 35: 205-217, 1962) in which he measured the effect of injecting different solutions on the rate of secretion of fishes swimming in a tank provide a method to search for clues of the nature of the variables. His studies on free swimming fish, however, have some limitations. For example, the frequency with which samples can be collected is restricted, thus distorting the determinations of the time course of any effect. In turn, the low frequency of sampling may also distort the assessment of secretion rates because a single sample collected during a long period may pool liquid produced while the gland was secreting at quite different rates. Finally, the same problems apply to the collection of samples of venous and arterial blood.

In this communication we describe experiments in anesthetized dogfish that allow us to follow closely the time course of the changes produced by different experimental modifications, Dogfish (Squalus acanthias) weighing between 2 and 3.8 kg. were injected in the caudal artery with nembutal (20 mg/kg). The gills were perfused at a rate of 2L/min. with sea water equilibrated with air (T = 15°C). After opening the abdomen with a midline incision, the rectum was opened near the insertion of the rectal gland. A polyethylene catheter was tied in the orifice of the rectal gland duct to collect continuously the secreted fluid. For intravenous injections, a fine needle (23 ga.) was introduced into the posterior intestinal vein. The NaCl solution infused into the animals was a shark Ringer solution in which all urea had been replaced with NaCl. The composition (in millimoles per liter) was NaCl,