the ability of isolated cells from large and small follicles of the skate Raja erinacea to produce steroids in the presence of steroid substrate in vitro is investigated.

Animals were sacrificed by spinal transection, and the ovaries removed and placed in cold isotonic buffer. After several washes, follicles were isolated, measured and subsequently punctured with scissors to release yolk. The follicular envelopes were then washed free of remaining yolk by rinsing in cold buffer, minced with scissors and dispersed with collagenase. Dispersed cells were washed and centrifuged at 800 xG for 5 minutes. Cells were finally reconstituted in basal medium containing urea, Eagle's salts and glutamine. Aliquots containing 250,000 cells were incubated in the presence or absence of steroid substrate for 4 hours at 20°C. Testosterone (T) and estradiol (E) content in the medium was later determined by radioimmunoassay.

In control incubates (without substrate), small amounts of T and E were produced, Table 1. Large follicles (> 10 mm) produced more T than small follicles (5-10 mm), and small follicles produced more E than large follicles. In the presence of steroid substrate, both T and E production were dramatically increased in a dose dependent manner. With progesterone as substrate, T production by cells from large follicles exceeded that by cells from small follicles. With T as substrate, cells from small follicles were more capable of producing E than cells from large follicles.

Table 1.--Synthesis of Radioimmunoassayable Testosterone and Estradiol By Skate Ovarian Follicular Cells in vitro. Testosterone (T) and Estradiol (E) production by follicular cells harvested from small (5-10 mm) and large (> 10 mm) follicles, and incubated with progesterone ($10^{-9}$ to $10^{-5}$M) or testosterone ($10^{-9}$ to $10^{-5}$M) substrate for 4 hours at 20°C

| T Production (pg/2.5 x $10^5$ cells) | | | | | | |
|---|---|---|---|---|---|---|
| Progesterone Substrate | C | $10^{-9}$ | $10^{-8}$ | $10^{-7}$ | $10^{-6}$ | $10^{-5}$ |
| Small Follicles | N.D. | 339 ± 47 | 877 ± 16 | 1158 ± 10 | 1030 ± 92 | 779 ± 42 |
| Large Follicles | 150 ± 14 | 653 ± 28 | 914 ± 60 | 6467 ± 793 | 19568 ± 1351 | 8132 ± 801 |
| E Production (pg/2.5 x $10^5$ cells) | | | | | | |
| Testosterone Substrate | C | $10^{-9}$ | $10^{-8}$ | $10^{-7}$ | $10^{-6}$ | $10^{-5}$ |
| Small Follicles | 519 ± 9 | 2629 ± 206 | 2817 ± 188 | 3184 ± 883 | 4250 ± 562 | 5230 ± 92 |
| Large Follicles | 120 ± 21 | 181 ± 12 | 478 ± 45 | 2038 ± 182 | 3413 ± 324 | 4443 ± 154 |

C - control; N.D. - non-detectable

These studies suggest that elasmobranch ovarian cells have steroidogenic potential, but that steroid synthesis appears to be highly substrate dependent. At this time, it is not clear whether this is a function of in vivo physiological state and a gonadotropin role in the regulation of endogenous substrate supply. Supported by NSF PCM 8104144 to IPC.

## A POSSIBLE ROLE FOR ESTRADIOL IN NIDAMENTAL GLAND FUNCTION

T.J. Koob, P. Tsang, J.J. Laffan and I.P. Callard, Department of Biology, Boston University and Department of Orthopaedic Surgery, Children's Hospital Medical Center, Boston, Ma.

With few exceptions and regardless of reproductive mode chondrichthyan females encapsulate eggs and albumin in morphologically complex shells made by the nidamental or shell gland located in the anterior reproductive tract. As eggs and albumen pass through the shell gland lumen, precursor egg case materials are secreted by glandular lamellae and deposited around the eggs and accompanying albumen. Egg case formation, however, seems not to require either the presence or passage of eggs since "egg-less" capsules have been recorded in at least two species (Richards et al., 1963, Bull Bing. Oc. Coll. 18, 5 - 65 in Raja erinacea; Templeman, W., 1944, Dept. Nat. Res. Nfld. Res. Bull. 15, 1 - 102 in Squalus acanthias). Since direct physical stimulation alone cannot explain normal deposition of shell materials, the stimulus for egg case formation must be sought elsewhere. We have begun examining the endocrine control of shell gland function, and this report describes preliminary data bearing on the possible involvement of estradiol in pre-spawning shell gland growth.

RESULTS--Thirty-two female Raja erinacea over 600 g total body weight were examined at Mount Desert Island Biological Laboratory. The presence of egg cases in the reproductive tract was noted, shell gland weights were determined and the diameter of the largest ovarian follicles was measured to the nearest mm with a calibrated template. A strong positive correlation was found between shell gland weight and follicle diameter (Figure 1, n = 62, correlation

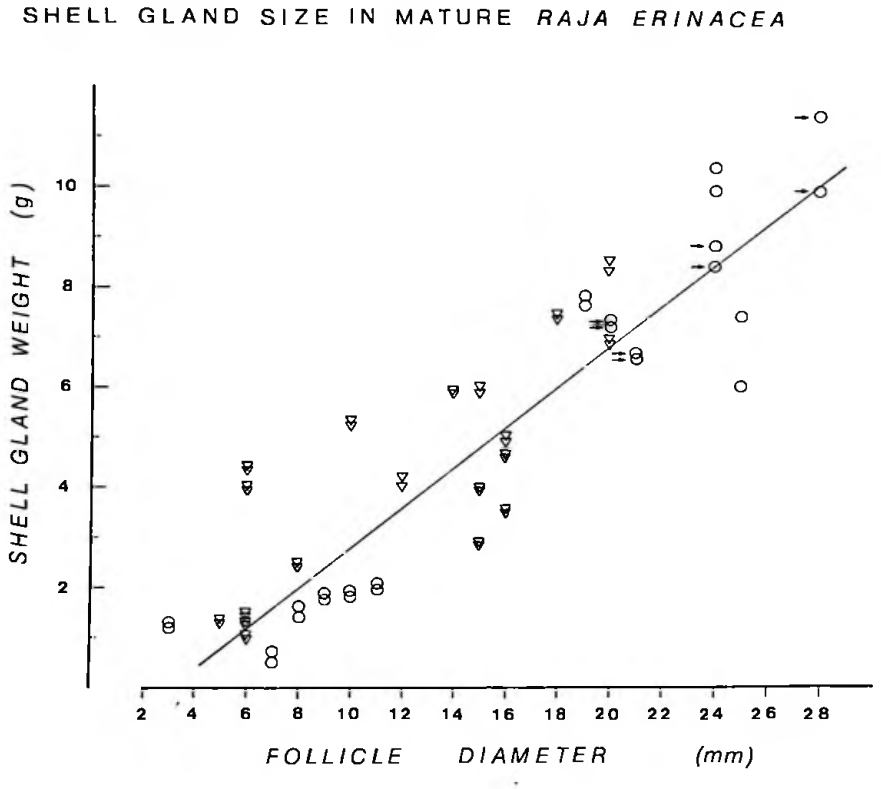


Figure 1.--Shell gland weight and follicle diameter in mature Raja erinacea.

coefficient r-.88) with a doubling of shell gland size for every 75% increase in follicle diameter. Only skates with follicles over 20 mm and shell glands over 6 g were found carrying egg cases (arrows, Figure 1). This represents at least a six-fold increase inggland size over that in resting females (follicles < 5 mm).

Circulating levels of 17-$\beta$ estradiol were measured by radioimmunoassay (see Tsang and Callard, 1981, The Bulletin 21, 55-56 for details of method) in serum collected at sacrifice from eight skates. Figure 2 shows the positive correlation found between 17-$\beta$ estradiol and follicle size (n=8, r=.69). When shell gland weights from these same skates were analyzed in relation to 17-$\beta$ estradiol, a positive correlation was found (n=8, r=.44). None of these skates were carrying eggs nor did any contain ovarian follicles over 20 mm, therefore, circulating estradiol titers during spawning remain to be measured.

DISCUSSION--After examining almost one thousand female Raja erinacea, from Block Island Sound, Richards et al (op. cit.) concluded that "both eggs and shell glands increased in size simultaneously." We have examined a small number of little skates from the Gulf of Maine and found the same positive correlation. Shell gland enlargement in preparation for spawning appears coincident with ovarian recrudescence and follicle growth. In addition, both follicles and shell glands must attain sufficient size before spawning will occur. Serum 17-$\beta$ estradiol titers also rise with follicle size suggesting that as follicles grow their steroidogenic capacity increases. Since shell

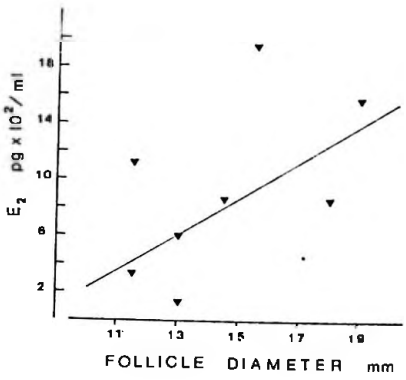


Figure 2.--Follicle diameter and serum 17-$\beta$ estradiol levels in female Raja erinacea.

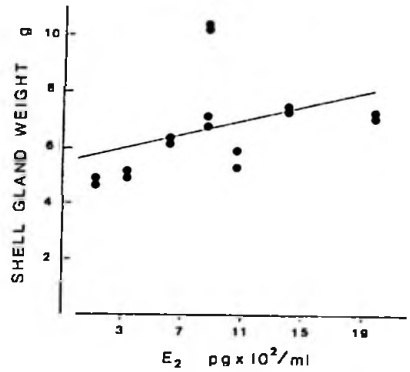


Figure 3.--Shell gland weight and serum 17-$\beta$ estradiol levels in pre-spawning Raja erinacea.

gland growth is coincident with both follicle enlargement and rising estradiol levels, it is likely that follicle derived estradiol influences shell gland function. We, as yet, have no direct evidence that estradiol can regulate nidamental gland function but are currently investigating this possibility. This work was supported by NSF PCM 81-04144 to I.P.C.

## THE EFFECT OF MAMMALIAN INSULIN ON PLASMA SUCROSE IN THE SKATE AND SPINY DOGFISH

John Laffan, Thomas J. Koob and Ian P. Callard, Department of Biology, Boston University, Boston, Ma.

Although comparative biochemical studies of vertebrate insulins indicate structural conservation, significant differences exist between mammalian and elasmobranch molecules (Steiner et al., Amer. Zool. 13:591-604, 1973). Nevertheless, mammalian insulin induces hypoglycemia in some elasmobranchs (Patent, Gen. Comp. Endocrinol. 14: 215-242, 1970). In this study we sought to characterize the temporal changes in blood sugar level (BSL) following a single intraperitoneal injection of bovine insulin (100 iu kg; Sigma). After injection of hormone or vehicle, blood samples were collected from the caudal aorta at 0.5, 3, 6, 12, 24 and, in the skate only, 48 hours post injection. Blood was centrifuged and plasma frozen until analysis by the glucose oxidase method (Worthington Diagnostics).

The results are shown in Table 1. Despite normal variability in starting values in a natural population each

TABLE I Percent change in blood sugar level (± SD) in Squalus acanthias and Raja erinacea following a single i.p. injection of bovine insulin (100 iu/kg)

| | Time (hours) | | | | | |
|---|---|---|---|---|---|---|
| | 0.5 | 3 | 6 | 12 | 24 | 48 |
| **Shark** | | | | | | |
| Insulin | -6.7±17.3 | -25.0±7.5 | -32.4±11.5 | -52.5±19.6 | -62.2±33.4 | - |
| Control | -4.9±16.3 | - 1.5±17.1 | 13.2±30.6 | 13.1±35.2 | 15.3±27.9 | - |
| p value | NS | <.025 | <.025 | <.01 | <.005 | |
| **Skate** | | | | | | |
| Insulin | -8.62±23.7 | -30.8±23.6 | -45.0±22.4 | -64.1±13.7 | -76.9±5.0 | -86.7±3.1 |
| Control | -7.96±9.9 | - 9.3±17.8 | -21.2±18.5 | 1.6±56.0 | -18.0±7.0 | 25.0±24.2 |
| p value | NS | <.10 | <.05 | <.05 | <.001 | <.001 |

* p values are given for comparison between injected and control animals at each time interval tested.

n = 5 in all instances.