temperature 250°C, column temperature 70°/20° per min/260°C, separator temperature 250°C, energy of bombarding electrons 22 eV. Repetitive magnetic scanning was carried out over the mass range 34-800 in 3 sec.

RESULTS AND DISCUSSION--The major component in the sulfate fraction had a retention index of 3740. The mass spectrum of the TMS ether derivative (Figure 1) showed a series of significant peaks at m/z 591, 501, 411

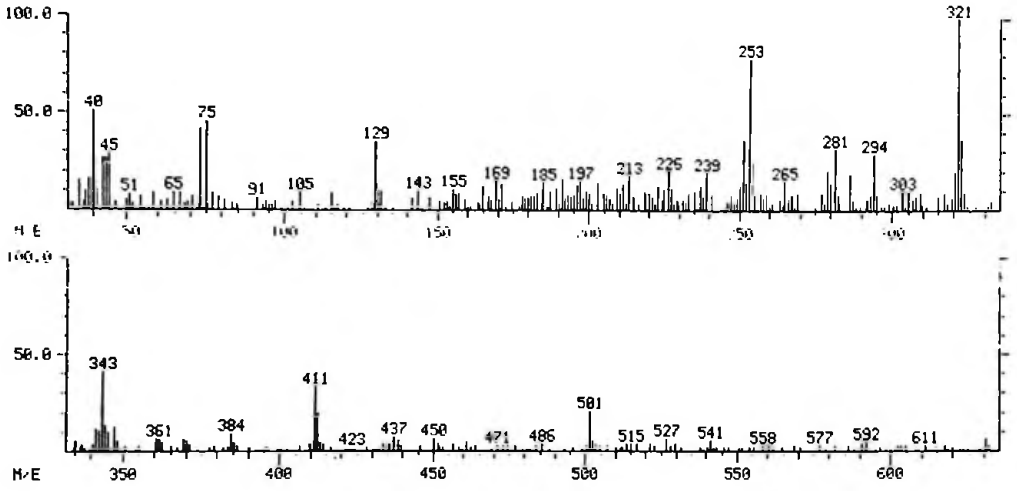


Figure 1.--Computer printout of the mass spectrum of the trimethylsilyl ether derivative of a bile alcohol isolated from skate bile at a retention index of 3740. The numbers shown above the peaks indicate mass fragments. Relevant "diagnostic ions" are reported in the text.

and 321 (base peak) formed by loss of 219 mass units (the 3 terminal carbons and 2 trimethylsilyl groups) and consecutive losses of trimethylsilanol from the molecular ion. In addition, peaks were found at m/z 631 (M-2x90-89), 541 (M-3x90-89), 450 (M-5x90), 360 (M-6x90) indicating a possible molecular weight of 900. Another series of peaks were found at m/z 617 (M-103-2x90), 527 (M-103-3x90); 437 (M-103-4x90) and 347 (M-103-5x90). Peaks at m/z 253 and 343 indicated three hydroxyl groups in the ring system. These findings are consistent with 5β-cholestane-3α,7α,12α,24,26,27-hexol (scymnol) but further studies are necessary for conclusive identification, i.e., microchemical reactions and chemical ionisation mass spectrometery.

In the analysis to date this highly polar sulfated bile alcohol, found for the first time in this species of skate, appears to be the dominant, if not exclusive, bile acid present in the bile. A search for other bile acids in the glucuronide fraction, for example, has proved fruitless. It is possible, therefore, that sulfated bile alcohol plays a major role in fat digestion and bile formation in the Small Skate that warrants further exploration. This investigation was supported by the Swiss National Science Foundation and the Sandoz-Stiftung zur Förderung der medizinisch-biologischen Wissenschaften. We are deeply indepted to Prof. Jan Sjövall for very helpful discussions. The technical assistance of Mr. Th. Weber and the secretarial help of Miss R. Steiner are gratefully acknowledged.

## STEROID PRODUCTION BY ISOLATED SKATE OVARIAN FOLLICULAR CELLS

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Elasmobranchs produce steroid hormones (Lance and Callard, The Vertebrate Ovary, Plenum Publishing Corp., 1978) and the ovaries are major sources of these hormones, since circulating levels of androgen and estrogen are markedly decreased after ovariectomy (Jenkins and Dodd, J. Fish Biol., 21:297, 1982). Previous studies have demonstrated steroidogenic capacity by use of radiolabelled precursors (Callard and Leathem, Arch. Anat. Microsc. Morphol. Exp., 54:35, 1965) and by enzyme histochemistry (Lance and Callard, Gen. Comp. Endo., 13:255, 1969). However, the specific cells responsible for steroid biosynthesis have yet to be definitively established. In this report,

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

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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.