The effect of endigin on binding of ^{3}H-ouabain to slices of rectal gland in the course of a 300 min incubation in Ringers solution was also tested (Bull. MDIBL, 21:103-108, 1981). Endigin (5×10^{-5}M) almost completely inhibited the binding of 10^{-9}M ^{3}H-ouabain, reducing it by 95%.

These experiments supply additional evidence that the endogenous non-steroidal digitalis-like compound found in anuran tissues may bind to ouabain receptors in the cell membranes of animal species widely separate in evolutionary development, and might therefore represent an example of an endogenous regulator of Na-K-ATPase.

MORPHOLOGICAL IDENTIFICATION OF STEROID PRODUCING CELLS IN THE TESTIS OF SQUALUS ACANTHIAS

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In previous studies we have demonstrated that the presence and activities of certain enzymes involved in the synthesis of both androgens and estrogens varied topographically in the testis of Squalus acanthias, according to the stage of spermatogenesis examined (Callard, G.V. and Petro, Z., The Bulletin, MDIBL 19:38, 1979; Callard, G.V. et al., The Bulletin, MDIBL 21:37, 1981). This implies that the steroidal microenvironment associated with these different phases of germ cell maturation may be quite specific. Light microscopic observations of the testis, at this time, indicated that the interstitial tissue, which in all vertebrate species so far studied contains the steroid-producing Leydig cells, appeared undifferentiated. The Sertoli cells, however, were well developed, which was taken as good evidence that these cells were responsible for the steroidogenic activity displayed by Squalus testis. To identify more precisely the cellular site of steroid production and to determine whether Leydig cells were present or absent in the testis of Squalus, tissue was also examined by means of electron microscopy.

The testes of pithed male sharks were perfused by cannulation of either the conus arteriosus or celiac artery. Perfusion was initiated by physiological saline containing 1% heparin which was allowed to flow for 10 mins. This was immediately followed by 500 ml of half-strength Karnovsky's fluid (Karnovsky, M.J., J. Cell Biol. 27:137A, 1965). Egress of the perfusate was accomplished by section of the atrium. The testes were then removed, sliced into small pieces and further fixed in Karnovsky's fluid for 24 hrs. Post-fixation was carried out by immersion in an aqueous 1:1 mixture of 2% osmium tetroxide and 3% potassium ferrocyanide for 1 hr. (Karnovsky, M.J., J. Cell Biol. 284A, 1971). The tissue was then processed for electron microscopy by conventional procedures.

Initial observations demonstrated that cells containing organelles known to be involved in the synthesis of steroids, e.g., smooth endoplasmic reticulum, lipid droplets, and mitochondria with tubulo-vesicular cristae, were present in the interstitial tissue of Squalus testis (Figure 1). These cells, however, were undifferentiated and possessed

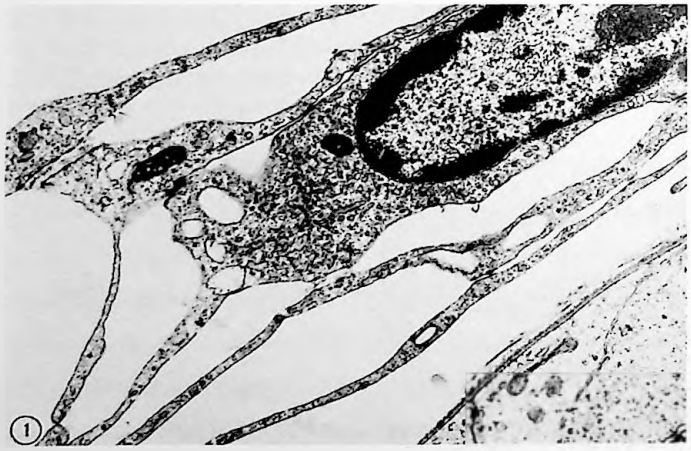

Figure 1.--Electron micrograph of "Leydig-like" cell present in the interstitial tissue of Squalus testis. These cells are fusiform in shape and produce long attenuated cytoplasmic processes. They are relatively undifferentiated and contain a few lipid droplets, mitochondria, and a small amount of smooth endoplasmic reticulum.

a small amount of smooth endoplasmic reticulum. This is the first report demonstrating that "Leydig-like" cells do occur in the interstitial tissue compartment of Squalus testis, although the exact analogy or homology of these cells with Leydig cells in the amniote testis is not yet proven. In contrast, the Sertoli cells contained a greater abundance of organelles associated with steroid production (Figure 2). These ultrastructural observations, plus the large volume of

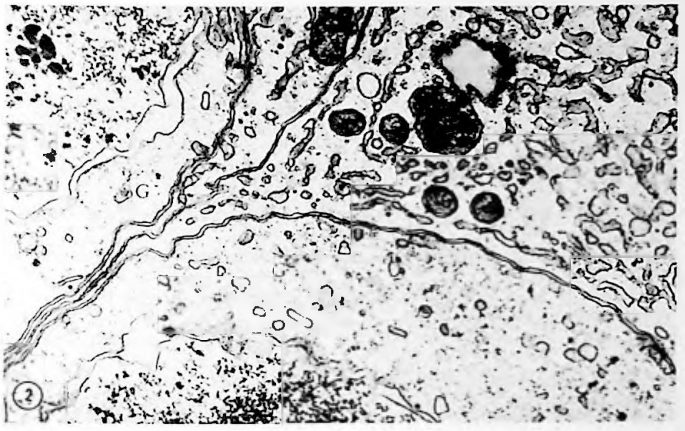

Figure 2.--Portion of a Sertoli cell surrounding two (G) germ cells (early spermatids). The Sertoli cells possess many lipid droplets, mitochondria and a large volume of smooth endoplasmic reticulum.

Sertoli cytoplasm present in the germinal epithelium support our earlier conclusion that these cells represent the major site of steroid synthesis in the testis of Squalus acanthias. Supported by NICHD-16715.

TRANSPORT OF β-ALANINE IN ISOLATED SKATE HEPATOCYTES

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It has been shown (Goldstein, J. Exp. Zool. 215:371-377, 1981) that the amino acid β-alanine plays a major role in the regulation of cell volume in the skate Raja erinacea. Adaptation of the skate to a dilute environment (50% sea water) leads to a release of β-alanine from muscle and erythrocytes reducing the intracellular solute concentration and hence limiting the entry of water into the cells. Unlike the other major amino acid involved, taurine, β-alanine in the plasma is eliminated via metabolism to CO_2 and water and the principal site of this oxidation is the liver. King et al. (J. Exp. Zool. 212:69-77, 1980) have shown that the oxidation of β-alanine in liver slices is 1.7 times faster in skate adapted to 50% seawater compared to those in normal seawater. This increased oxidation could be achieved by modifications at the enzyme level and/or by changes in the transport of β-alanine into the liver cells. Leach et al (Bull. MDIBL 21:64-66, 1981) could detect no changes in the activity of the relevant enzymes (β-alanine transaminase and malonate semialdehyde dehydrogenase) on keeping skate in 50% seawater but did point out that, as the K_m for the transaminase (1.11 mmol ℓ^{-1}) was very close to the concentration of β-alanine in the liver tissue (0.97 mmol ℓ^{-1}), it was likely that the flux through this pathway would be very sensitive to substrate availability, and hence to the rate of β-alanine transport into the liver cells.

The purpose of the study described was to attempt to isolate viable hepatocytes from the liver of the skate, Raja erinacea, and use these to investigate whether the transport of β-alanine into the cells of the liver is a potential site for the regulation of the overall metabolism of the amino acid in vitro.

Method. Fish were caught in Frenchman Bay and kept in tanks containing running seawater until use. For those specimens adapted to 50% seawater, this was achieved by progressive dilution over six days and the fish were held at this salinity for a minimum of two days before use. Fish were killed by pithing of the brain and spinal cord, opened ventrally and 60 mℓ of ice-cold Ca^{++}/Mg^{++}-free skate saline (= CMFS; adapted from King et al, J. exp. Zool. 212: