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

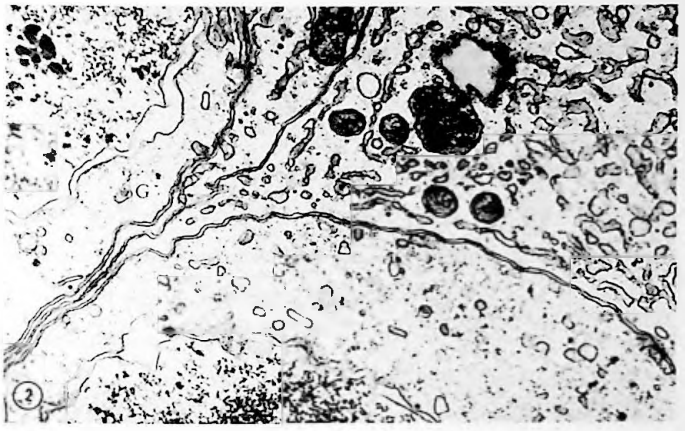

Figure 2.--Portion of a Sertoli cell surrounding two (G) germ cells (early spermatids). The Sertoli cells possess many lipid droplets, mitochondria and a large volume of smooth endoplasmic reticulum.

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:

69 - 77, 1980, with the addition of 10 mmol ℓ^{-1} Hepes) was rapidly perfused through the hepatic portal vein. The liver was removed, placed in ice-cold CMFS and chopped into approximately 1 cm cubes with scissors. The tissue were then finely minced over ice using a razor blade, transferred to 25 mℓ of 0.1% collagenase in CMFS and stirred slowly (approx. 80 rpm) for two hours at 15°C. The dispersed tissue was then poured through gauze and the filtrate collected. Hepatocytes were isolated from the filtrate by differential centrifugation to remove dispersed fat, pigment cells and any remaining erythrocytes. The cells were then washed twice in CMFS and finally once in normal skate saline plus 0.1% bovine serum albumin (BSA).

Aliquots of dispersed hepatocytes were added to the appropriate saline + BSA containing 0.1 μCi $m\ell^{-1}$ ^{14}C-β-alanine and 0.5 μCi $m\ell^{-1}$ ^{3}H-polyethylene glycol (MW 4000) to permit correction for extracellular space. All incubation media also contained 2.0 mmol ℓ^{-1} amino-oxyacetate to prevent the ^{14}C-β-alanine taken up by the cells from being metabolished (Leach et al., loc. cit.). All incubations were in an atmosphere of 99% O_2:1% CO_2, in a shaking water bath at 15°C. After incubation the cell suspensions were centrifuged and samples of the supernatant and the pellet, after digestion, taken for double isotope (^{14}C and ^{3}H) liquid scintillation analysis with appropriate correction for "spillover", following the method of Kobayashi and Maudsley, Biological Applications of Liquid Scintillation Counting, Academic Press, 1974.

Results and Discussion. Microscopic examination of the cell suspensions obtained showed relatively clean preparations of isolated hepatocytes (Figure 1). The principal contaminant, when present, was pigment cells. The hepatocytes were rounded, contained numerous oil droplets and showed a high percentage viability when tested with trypan blue. The hepatocytes took up β-alanine from an external concentration of 0.1 mmol ℓ^{-1} in a linear fashion for periods in excess of 150 minutes, concentrating the amino acid inside the cells to levels up to twelve times that in the external medium. At an external β-alanine concentration of 0.1 mmol ℓ^{-1} the rate of uptake in hepatocytes from fish kept in normal seawater was 0.40 ± 0.05 μmol g^{-1} h^{-1} (mean ± S.E., N = 11). In the presence of 1.0 mmol ℓ^{-1} p-chloromercuribenzoate, uptake was reduced to 18.5 ± 11.7% (N = 3) of control values, indicating that the uptake was influenced by membrane sulphydryl groups. Measurement of the uptake at a range of external β-alanine concentrations revealed a hyperbolic relationship (Figure 2) suggesting saturation of the transport system at high β-alanine concentrations. Double-reciprocal analysis yielded the following parameters for the uptake process: K_m = 0.17 mmol ℓ^{-1}, V_{max} = 1.51 μmol g^{-1} h^{-1}. It should be noted that typical plasma concentrations of β-alanine in R. erinacea are 0.1 - 0.2 mmol ℓ^{-1}.

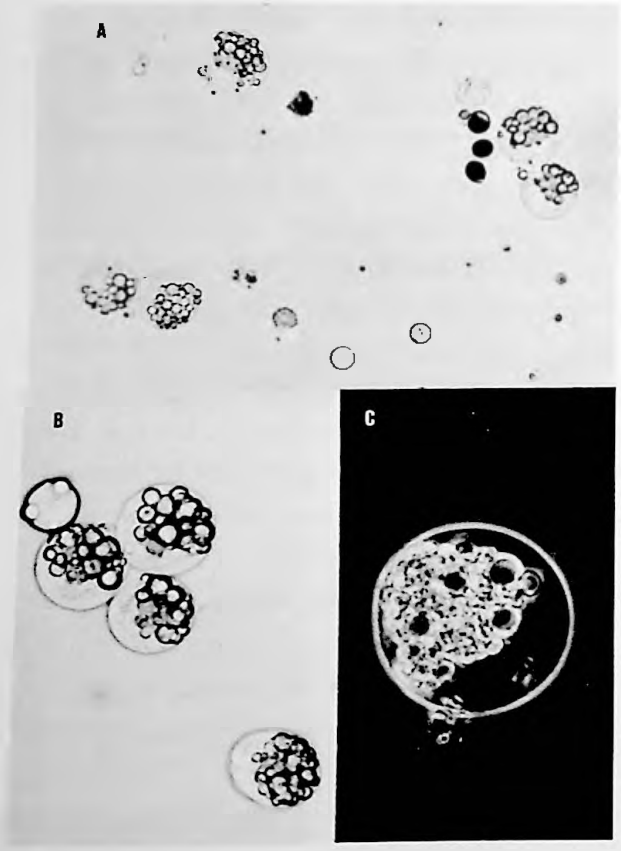

Figure 1.--Light microscope views of isolated hepatocytes from the skate. A x 500; B x 625; C x 1250 (with dark field illumination).

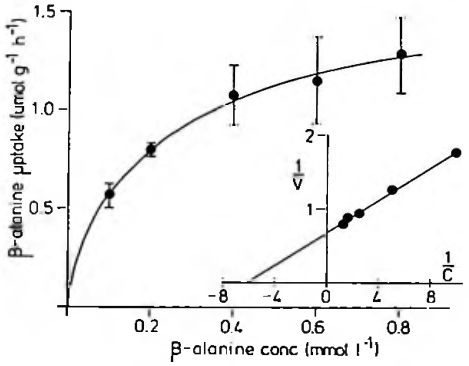

Figure 2.--Rate of β-alanine uptake at different external concentrations. All values mean ± S.E. (N = 3). Inset : same data on a double-reciprocal plot.

Preliminary investigations of the specificity of the transport system for β-alanine were also made. In the presence of a 200-fold excess of L-alanine or taurine the uptake of β-alanine was unaffected (Table 1). However, a similar excess of γ-amino butyrate (GABA) resulted in a 75% inhibition of the simultaneous uptake of β-alanine.

TABLE 1.--Effect of amino acids on β-alanine uptake (β-alanine conc. = 0.1 mmol ℓ^{-1}). N=3 in all cases

Treatment	Control	Experimental	
L-alanine (20 mmol ℓ^{-1})	0.47 ± 0.05	0.41 ± 0.02	N.S.
taurine (20 mmol ℓ^{-1})	0.47 ± 0.05	0.48 ± 0.06	N.S.
GABA (20 mmol ℓ^{-1})	0.46 ± 0.06	0.11 ± 0.04	$p < 0.01$

The effect of adaptation to 50% seawater on the β-alanine transport kinetics in isolated hepatocytes was investigated and the results are given in Table 2. Hepatocytes isolated from skate adapted to 50% seawater were

TABLE 2.--Comparison of β-alanine uptake in hepatocytes from skate from 100% SW and 50% SW. Mean ± S.E. (N)

	Control (100% SW)	Diluted (50% SW)	
Uptake at 0.1 mmol ℓ^{-1}	0.34 ± 0.07 (4)	0.32 ± 0.02 (5)	N.S.
Uptake at 0.8 mmol ℓ^{-1}	1.06 ± 0.42 (5)	0.89 ± 0.16 (6)	N.S.
Calculated K_m	0.19	0.28	
Calculated V_{max}	1.50	1.20	

incubated in saline with reduced NaCl (to 200 mmol ℓ^{-1}) and urea (to 270 mmol ℓ^{-1}) concentrations in order to mimic their reduced concentrations in the plasma. Adaptation of the skate to 50% seawater did not significantly change the rate or transport kinetics of β-alanine uptake in the isolated hepatocytes.

If the activities of the enzymes involved in β-alanine metabolism (Leech et al, loc. cit.) and the transport kinetics for β-alanine uptake are unchanged in fish adapted to 50% seawater how, then, can the increase in β-alanine oxidation, both in vivo and in incubated liver slices, upon adaptation to a dilute environment, be explained? The K_m for the uptake of β-alanine was found to be 0.17 mmol ℓ^{-1}, a value within the normal range of plasma concentrations of the amino acid. In addition, Leech et al, found that the K_m for the transaminase in-

volved in β-alanine metabolism was very close to the actual concentration of the amino acid in the liver. It therefore seems likely that the overall oxidation of β-alanine in the liver would be very sensitive to changes in the intracellular concentration of the amino acid, at least partly determined by the uptake rate which would itself be very sensitive to changes in the concentration of β-alanine at the transport site. In the incubated liver slices used by King et al, the medium β-alanine concentration was held constant at 0.1 mmol ℓ^{-1}. In this case, the much expanded extracellular space in tissue from skates adapted to 50% seawater may be expected to pose less of a restriction on the access of β-alanine in the incubation medium to all the available transport sites as compared to that in the tissue from fish kept in normal seawater. Hence the physical characteristics of the tissue, when incubated as a slice, may have led to changes in the actual concentration of β-alanine at the transport site and hence, for the reasons described above, to oxidation rate. In vivo small increases in plasma β-alanine concentration may be expected to produce relatively large increases in the oxidation rate of this amino acid in the liver and may thus form the basis of an increased metabolism of β-alanine by the liver of skate on exposure to a dilute environment. This work was supported by grants from the Nuffield Foundation (to T.J.S.) and Whitehall plus National Science Foundations (to L.G.).

CRYOGENIC ACTIVITY IN THE URINE OF DOGFISH SHARK, SQUALUS ACANTHIAS

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The urine of human beings, rabbits, and dogs contains a substance that lowers body temperature (Herringham, J. Pathol. Bacteriol. 6:158, 1900; Kluger et al, Am. J. Physiol. : R271, 1981). This endogenously produced cryogenic substance, "endogenous cryogen" (EC), results in a regulated fall in body temperature. Injection of EC into rabbits results in peripheral vasodilation, a decrease in metabolic heat production, and an increase in respiratory rate. During heat stress the amount of EC that is excreted by resting human subjects decreases (Kluger et al, Fed. Proc. 41:976, 1982). We hypothesized that this decreased excretion results in an increased plasma concentration of EC, and as a result, part of the thermoregulatory responses designed to lower body temperature during heat stress could be triggered by this increase in EC. It is also possible that many cyclical changes in body temperature (e.g. circadian changes, cyclical fevers, etc.) are regulated by the internal concentration of EC.

Other than the appearance of EC in the urine of the mammals listed above, nothing is known about the comparative biology of EC. For example, do nonmammalian vertebrates produce and excrete a cryogen in their urine? In this report we present preliminary data indicating that the urine of the dogfish shark contains a substance that lowers deep body temperature and induces peripheral vasodilation in rabbits.

MATERIALS AND METHODS--Experimental Animals. Female sharks, Squalus acanthias, weighing 3.4 to 6.8 kg were collected off the coast of Maine and maintained in fresh seawater at 14 to 16°C at the Mount Desert Island Biological Laboratory. To bioassay for the presence of EC, male New Zealand white rabbits weighing approximately 2.8 to 3.3 kg were used in these studies. The rabbits were maintained on a diet of Teklad rabbit chow and water ad libitum.

Urine Collection.--Large balloons (Smurf) were washed 10 times with tap water and then 3 times with sterile pyrogen-free 0.9% sodium chloride. A piece of polyethylene tubing (ca. 15 cm) was inserted approximately 3 cm into the opening of the balloon and tied in place with cotton thread. To insert the free end of the catheter into the urinary tract each shark was held out of the water by its tail with the anterior 1/3 still in the holding tank. The distal 3 mm of the renal papilla was cut and the catheter inserted about 3 cm and tied in place. The catheter was also sutured to the tail of the shark about 5 cm posterior to the renal papilla, and the shark released into the holding