HDL would foster the efficient removal of tissue cholesterol and its subsequent elimination from the body by the liver and, by contrast, low levels of HDL could lead to excessive accumulation of cholesterol in tissue. Additional studies are needed to delineate the mechanism of hepatic uptake of free cholesterol, and to determine whether other lipoproteins contribute substantially to the bile acid and biliary cholesterol hepatic precursor sites, and whether one particular HDL subfraction is more active than another.

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Tunicate Eggs Utilize Ammonium Ions for Flotation

Abstract. *Unlike most solitary ascidians,* Corella willmeriana *retains its eggs and embryos well past hatching. The early stages float to the top of the enlarged atrium from which they cannot escape. Ammonium ions replace other more dense substances in the cell sap of the float cells surrounding the embryo. Energy derived from glycolysis but not mitochondrial processes supports this process.*

Among the many different adaptations to flotation in the marine environment, the substitution of ammonium ions for more dense substances is at present known to occur only in certain phytoplankton cells (*1*) and a few pelagic cephalopods (*2*). *Corella willmeriana*, from the U.S. Pacific Northwest, is one of the few ascidians that produce floating eggs. In all ascidians the eggs and embryos are surrounded by two cellular layers and a noncellular chorion. The outer cellular layer consists of follicle cells that are especially large in *C. willmeriana* and in the European species *C. parallelogramma* and *Ascidiella aspersa*, which also have floating eggs (*3*). In *C. willmeriana* these cells are responsible for egg flotation; if they are removed, the eggs rapidly sink (*4*). The floating eggs are an adaptation to brooding, as they are incubated in an enlarged atrial chamber in which the siphonal opening is never uppermost. After hatching the tadpoles swim actively upward, which may ensure their retention in the atrium until they are quite advanced, an optimal strategy for a fugitive species such as *C. willmeriana* (*5*). We have determined, by chemical analysis, that the low density of the follicle cells results from the fact that ammonium ions replace other, more dense elements of the vacuolar sap. Inhibitor studies implicate glycolysis but not mitochondrial processes in providing the energy to maintain the asymmetry of ammonium concentration across the thin peripheral cytoplasm of the follicle cells.

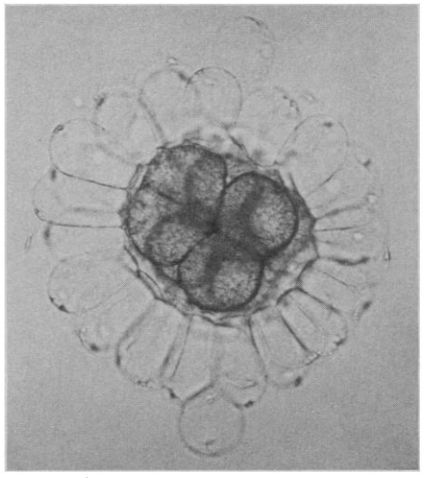

Fig. 1. Embryo of *Corella willmeriana*, showing large flotation cells surrounding the chorion.

Each embryo is surrounded by an average of 89 flotation cells which are 65 μm in diameter (Fig. 1), giving a flotation volume of 1.31×10^{-5} cm^3 per embryo. The chorion has a diameter of 170 μm with an enclosed volume of 2.57×10^{-6} cm^3, yielding a fivefold excess of flotation space to embryonic space. Naturally spawned eggs float in seawater with a salinity of 28.0 per mil (density, 1.023 g/cm^3) and in dilutions of seawater with salinities as low as 19.5 per mil (calculated density, 1.02 g/cm^3). However, the eggs rise at a rate of 2.41×10^{-2} cm/sec in seawater with a salinity of 28.0 per mil, giving a calculated density of 1.018 g/cm^3 (*6*). Eggs and embryos that are deprived of their float cells as a result of being shaken in a test tube were found by isopycnic centrifugation in sucrose-seawater solutions to have a density of about 1.08 g/cm^3. Thus the follicle cells decrease the density by about 0.06 g/cm^3 in the intact embryo.

The follicle cells are composed of a thin rind of cytoplasm with a single large central vacuole; obviously, the vacuolar contents are responsible for the observed decrease in density. One appealing possibility would be that the follicle cells are filled with lipid, which would make it possible for them to float. Since formalin-fixed eggs sink rapidly, lipids are probably not involved. In addition, the lipophilic stain sudan black B fails to stain the vacuolar sap. Since ammonium ions have been implicated in the flotation of other marine forms, it seemed reasonable to analyze the ammonium content of the follicle cells. We shook eggs and embryos gently in test tubes to remove the follicle cells. The embryos were allowed to settle; then the follicle cells in the supernatant were lysed, and we determined their ammonium content by the colorimetric analysis of indophenol blue produced from ammonia with commercially prepared reagents (Boehringer Manheim). The follicular fraction contained large amounts of ammonium. However, no ammonium ions were detected in the eggs and embryos. By counting the number of embryos extract-

Table 1. The effect of metabolic inhibitors on the flotation of *Corella* eggs.

Inhibitor	Concentration (m*M*)	Effect on	
		Flotation	Development
Sodium azide	3	Float	Inhibits
Dinitrophenol	2	Float	Inhibits
Malonic acid	1	Float	
Sodium fluoride	3	Sink	Supports
Iodoacetic acid	3	Sink	Inhibits

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ed and knowing the flotation volume per embryo and the total quantity of ammonium, we were able to determine that the ammonium concentration of the vacuolar sap is 346 ± 38.6 m*M* (standard error of the mean) ($N = 5$).

Since the ammonium concentration of seawater is negligible, there exists a tremendous asymmetry of ammonium across the peripheral cytoplasm of the follicle cells. To support this gradient, metabolic energy must be expended. Metabolic inhibitors were used to determine the source of the energy (Table 1). The mitochondrial inhibitors sodium azide, dinitrophenol, and malonic acid had no effect on flotation after 10 hours of exposure even though development was arrested. The inhibition of glycolysis by sodium fluoride or iodoacetic acid, however, caused the eggs to gradually sink to the bottom after 4 or 5 hours. Thus, glycolysis is necessary for flotation. The gradual sinking of the cells suggests either that the inhibitors penetrate very slowly or that there is a store of energy sufficient for this length of time contained within the cells.

Ascidians are the second group of metazoans demonstrated to use ammonium ions for flotation. Pelagic squids have flotation chambers with twice the volume of tissue space and have ammonium concentrations of 480 m*M* (*6*), whereas *Corella* has a lower ammonium concentration (346 m*M*) but a greater ratio of flotation to tissue space (5:1). The source of the ammonium for flotation in ascidian eggs is presently unknown. *Corella willmeriana* excretes about half the ammonia (on a gram-hour basis) of similar ascidians (*7*) but this species also sequesters uric acid (*8*), and so strict comparisons are difficult. The fact that such distantly related organisms as algae (*1*), mollusks (*2*), and chordates use ammonium for part of their flotation requirements demonstrates that it is efficient enough to be more widespread than previously thought.

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Immunoassay of Androgen Binding Protein in Blood: A New Approach for Study of the Seminiferous Tubule

Abstract. *Androgen binding protein, a secretory product of seminiferous tubules, was isolated by means of affinity chromatography. A radioimmunoassay was developed and used to identify androgen binding protein in rat plasma. The ability to measure a testicular protein in blood provides a new method for investigation of seminiferous tubular physiology.*

The testis can functionally be divided into the Leydig cell and the seminiferous tubular compartments which produce, respectively, androgens and a complex fluid containing spermatozoa and various proteins. The study of the Leydig cell compartment was greatly facilitated by development of assays for measuring testosterone and other androgens in blood (*1*). By contrast, the physiology of the seminiferous tubule cannot be examined as easily since its products are released into the tubular lumen which is behind the "blood-testis barrier." This barrier, which is formed by Sertoli cell junctional complexes, limits entry of tubular contents into blood (*2*). Until recently, the only easily measurable product of the seminiferous tubular compartment was germ cells which were quantified either in the ejaculate or on histological sections of testicular biopsies. Studies of the seminiferous tubular compartment were improved by the identification of androgen binding protein (ABP), a secretory product of Sertoli cells (*3*). Although ABP comprises only 0.01 percent of total testicular protein, it can be assayed because of its ability to bind [^{3}H]androgens. Several studies showing a decline in ABP after hypophysectomy and an increase after the administration of follicle stimulating hormone (FSH) and testosterone indicate that ABP reflects the hormonal responsiveness of Sertoli cells (*4*).

Androgen binding protein is secreted into the lumen of the seminiferous tubules and transported to the epididymis where it is either inactivated or degraded (*5*). Since ABP is considered to be secreted behind the blood-testis barrier, it was not thought to enter the blood. This assumption was supported by the inability to measure ABP in plasma with conventional binding assays (*6*). As a consequence, studies of seminiferous tubular physiology which employ androgen binding to quantify ABP still require testicular biopsies.

Our initial interest in ABP was stimulated by the need for a more sensitive assay. The synthesis of a novel androgen affinity column (*7*) provided a technique for isolation of homogeneous rat ABP. A radioimmunoassay was developed and used in the present experiment to identify ABP in rat plasma.

Androgen binding protein was isolated from rat epididymides by sequential ammonium sulfate precipitation, androgen affinity chromatography, gel filtration, and ion exchange chromatography. These fractionation procedures produced an ABP preparation, purified 5000-fold over the crude homogenate, with an overall recovery of 45 percent of the initial binding activity. This preparation, which appeared homogeneous on native and sodium dodecyl sulfate containing polyacrylamide gels (*7*), was used to generate high-titer specific antibodies in female New Zealand White rabbits (*8*). Homogeneous APB was labeled with ^{125}I (specific activity, 40 to 60 μCi/μg) by the chloramine-T method (*9*), purified by ion exchange chromatography (*10*) and used to demonstrate immunoreactive ABP in tissue (Fig. 1). The ABP in both testicular and epididymal extracts as well as the homogeneous protein reacted identically with two different antiserums. Unexpectedly, immunoreactive ABP was also found in the plasma of male, but not female, rats (Fig. 1). Although ABP concentration in plasma is low (1.5 percent of epididymal tissue concentration), the amount of this protein in the total blood volume of the rat is equivalent to 15 percent of epididymal or 45 percent of testicular content. Tissue extracts from kidney, liver, and spleen of female rats had less than 1 percent of the cross-reactivity of testicular preparations.

Since a seminiferous tubular protein

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