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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9. We thank R. R. Strathmann and A. O. D. Willows for their hospitality in making the facilities of the Friday Harbor Laboratories available to us. K. Banse and D. Fox gave several valuable suggestions in the early part of this work. We also thank D. Fromson and M. Horn for criticism of the manuscript.

1 November 1977; revised 9 January 1978

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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had not, to our knowledge, been previously identified in blood, it was important to determine whether the immunoreactive material in male plasma possessed the same properties as ABP in the reproductive tract. To assess molecular size, plasma from male rats was fractionated on a calibrated Sephadex G-200 colume. Immunoreactive ABP was eluted with the same partition coefficient as the homogeneous protein (*7*) and the ABP in testicular and epididymal extracts assayed by androgen binding (*11*). The ability of circulating ABP to bind androgens was assessed by application of 1 ml of plasma to an androgen affinity column (1-ml bed volume) (*7*). Greater than 98 percent of the immunoreactive ABP was bound to the affinity resin and only 5 percent was eluted when the bulk of the protein was removed with five volumes of 1.0*M* KCl. However, 1 ml of buffer containing dihydrotestosterone (70 μM, 17β-hydroxy-5α-androstan-3-one) displaced 70 percent of the immunoreactive material from the affinity column, a recovery comparable to that observed during the isolation of epididymal ABP (*7*).

The testicular origin of plasma ABP was confirmed by demonstrating the exponential decline of this protein after orchiectomy (Fig. 2). The half-life ($t_{1/2}$) for disappearance was 1 day, and by day 7, ABP was undetectable. The ABP "secretion rate," estimated from the disappearance curve and the plasma concentration, is equivalent to 20 percent of total testicular content per day. Finally, to ensure that immunoreactive plasma ABP was not an androgen inducible protein made in an extragonadal site, castrated male and intact female rats were treated with testosterone propionate (7 days; 1 mg/day, subcutaneously). This treatment failed to maintain plasma ABP levels in castrated male animals and did not induce the protein in females.

Our findings provide direct evidence that a seminiferous tubular protein is released into the blood. Since ABP is in high concentration in both testis and epididymis, it could enter the blood from either of these organs. Studies suggest, however, that the plasma ABP concentrations correlate with testicular rather than epididymal levels after both hypophysectomy and androgen replacement therapy (*12*). These last observations are consistent with the hypothesis that ABP is released into blood from the testis rather than in some other part of the male reproductive tract. As a consequence, plasma ABP measurements may be useful in studying the physiology of the seminiferous tubular compartment. In addition, plasma ABP assays provide a method for longitudinal studies of gonadal disorders (*11, 13*) which will complement testicular biopsy. In species other than rat the study of ABP has been hampered by testosterone-estradiol binding globulin (TeBG), an androgen binding protein in blood. This TeBG is physically similar to ABP in rabbit (*14*) and man (*15*), and in rabbit these proteins may share common antigenic determinants (*16*). The present study provides added impetus to distinguish TeBG and ABP in the blood of man.

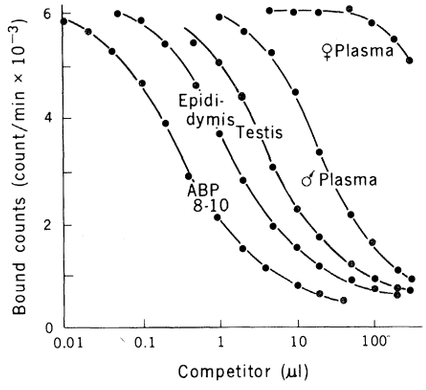

Fig. 1. Displacement of ^{125}I-labeled ABP from antiserum to ABP by: homogeneous ABP preparation 8-10 (4.2 μg/ml); 105,000*g* supernatants of epididymal (1 : 9, weight to volume) and testicular (1 : 2, weight to volume) homogenates; and plasma. Samples were brought to 0.3 ml with phosphate-buffered saline (0.01*M* sodium phosphate, 0.15*M* NaCl, *p*H 7.8). Antiserum to ABP (0.1 ml of 1:2000 working dilution) and ^{125}I-labeled ABP (0.1 ml, 100,000 counts per minute per milliliter) were added and the mixture was incubated for 4 days at 4°C. Bound counts were precipitated with goat antiserum to rabbit gamma globulin and counted. The graph shows bound counts plotted against microliter equivalents of the above ABP preparations.

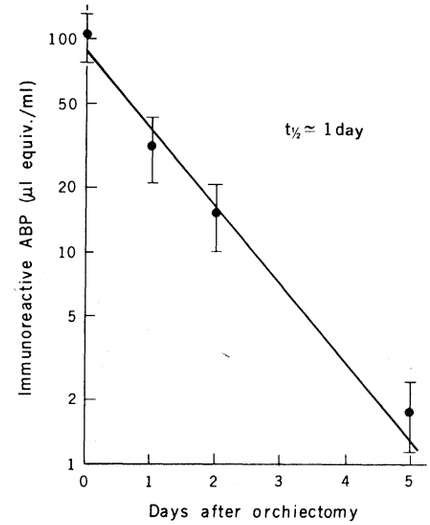

Fig. 2. Disappearance of immunoreactive ABP from plasma of male rats subjected to orchiectomy. Plasma was assayed for immunoreactive ABP as described in Fig. 1. The ABP content is expressed as microliter equivalents of epididymal cytosol [105,000*g* supernatant of 1:9 homogenate prepared from mature rat (275 g) epididymides] per milliliter of plasma (mean ± standard deviation; N = 3).

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17. We thank P. Gunsalus for protein iodination and A. Scanlin Tavani for technical assistance. This research was supported in part by USPHS grants HD 05276 and HD 09542.

14 November 1977; revised 3 January 1978