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EFFECT OF ALBUMIN ON PROLUMINAL MOVEMENT OF ^{3}H-ANDROGEN INTO SEMINIFEROUS AND EPIDIDYMAL TUBULES AND ANDROGEN BINDING IN THE INTERSTITIUM OF THE TESTIS AND EPIDIDYMIS AFTER PERIFUSION WITH FLUID CONTAINING ALBUMIN

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ABSTRACT

Effect of albumin on proluminal androgen movement from the peritubular space to the intratubular fluids of the adult rat testis and epididymis was examined by using in vivo microperifusion and subsequent micropuncture of the seminiferous tubules and caput epididymal tubules. Tubules were perifused with four different fluids: (1) Minimum Essential Medium (MEM) containing ^{3}H-testosterone and ^{14}C-polyethyleneglycol (PEG) alone; (2) MEM + 8 mg/ml Bovine Serum Albumin (BSA) containing the same radiolabeled compounds as above; (3) MEM + 80 mg/ml albumin containing the same radiolabeled compounds as above; and (4) Testosterone-free rat serum containing the same radiolabeled compounds as above. Bound ^{3}H-androgens in the interstitial fluids of the testis and epididymis after one-hour perifusion with the four different fluids above were measured by charcoal assay. In the testis, proluminal ^{3}H-androgen movement was not significantly altered by addition of albumin to the perifusion fluid ($p = 0.08$). Bound ^{3}H-androgens in the interstitial fluid after perifusion were significantly increased with increasing albumin concentrations in the perifusion fluid. In the caput epididymis, proluminal ^{3}H-androgen movement was significantly decreased with increasing albumin concentration in the perifusion fluid. Bound ^{3}H-androgens in the interstitial fluid after perifusion were significantly increased with increasing albumin concentrations in the perifusion fluid ($p<0.05$). These findings suggest that proluminal transepithelial movement of ^{3}H-androgens in the reproductive tract could be influenced by the presence of albumin, androgen-binding protein or some other binding protein in the peritubular space.

Key Words: Androgen binding, Androgen movement, Testis, Epididymis, Micropuncture

INTRODUCTION

The testis and epididymis are androgen target tissues that provide an important microenvironment for spermatogenesis, sperm maturation and epididymal metabolism or epididymal epithelial secretion;[1,2] nevertheless, very little is known about local mechanisms that control transepithelial movement of androgen in seminiferous and epididymal tubules.

Recently, a new system of in vivo microperifusion and subsequent in vivo micropuncture was developed to examine the movement of ^{3}H-androgens from blood to lumen and from interstitium to lumen in the rat testis and epididymis.[3,4] Movement of ^{3}H-androgen into the seminiferous tubule lumen was restricted with intraluminal androgen concentrations plateauing by one hour at approximately 10% to 15% of the extratubular androgen concentrations whether the

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^{3}H-androgen originated in the vascular or interstitial space.[3] Additionally, androgen movement from the interstitium to the lumen in the testis was not altered by a 10-times higher concentration of ^{3}H-testosterone in the perifusion fluid.[5] In the caput epididymis, intraluminal ^{3}H-androgen plateaued at approximately 35% of the vascular ^{3}H-androgen concentrations, but when ^{3}H-androgens were perifused around the tubules, movement of ^{3}H-androgen occurred against a concentration gradient.[3,4] This uphill movement was subject to competitive inhibition with 10 times and 100 times the concentration of ^{3}H-testosterone.

The importance of these findings is that there is a fundamental difference in androgen movement across the seminiferous and epididymal epithelia. The mechanism underlying this difference may involve differences in androgen binding to some protein in the interstitial space. Physiological studies have indicated that there is a substantial concentration of serum albumin in the interstitial tissue of the rat testis.[6] Testicular interstitial fluid exhibits essentially the same concentration of albumin as is found in serum.[6] Since serum albumin is the principal carrier protein for testosterone in the plasma and interstitial fluid of rats,[12] its abundance in the interstitial tissue space could influence proluminal androgen movement. The present study was undertaken to determine whether or not androgen binding to albumin or some other protein in the interstitial compartments of the testis and epididymis affects movement of ^{3}H-androgen into the tubules of those organs.

MATERIALS AND METHODS

In Vivo Microperifusion and Micropuncture in the Testis and Caput Epididymis

Adult male Sprague-Dawley rats (530 to 670 gm) were maintained in a constant temperature (25°C) vivarium under a 12-h light/12-h dark cycle and had free access to food and water. Animals were anesthetized and prepared for in vivo perifusion and micropuncture of reproductive tract tubules, as previously described.[3,4] Briefly, a testis and an epididymis of each animal were exteriorized in a special testicle holder and stabilized in 33°C, 2% agar. A small window in the agar to the superior surface of the testis or epididymis was left open and covered with mineral oil. The tubules were perifused in vivo through a sharpened, 100-μm tip micropipette inserted through the tunica albuginea. The tip of the pipette was always kept in sight to ensure that the seminiferous or epididymal tubule was not cut or punctured. The perifusion pump was set for a priming infusion at a rate of 36 μl/min in the testis and 6 μl/min in the caput epididymis for 15 min. Subsequently, a sustaining perifusion rate was set at 6 μl/min in the testis and 2 μl/min in the caput epididymis for the remainder of the experiment.

Four different perifusion fluids were prepared: (1) Lissamine-green-dyed Minimum Essential Medium (MEM) containing 26.7 μCi ^{3}H-testosterone (New England Nuclear, Boston, MA; specific activity: 55.2 Ci/mmol)/ml; 480 nM testosterone and 1.3 μCi ^{14}C-polyethyleneglycol (PEG, New England Nuclear; specific activity: 15 mCi/g)/ml; control fluid; (2) MEM + 8 mg/ml Bovine Serum Albumin (Sigma Chemical Company, MO, BSA) containing the same radiolabeled compounds as above (8 mg/ml BSA fluid); (3) MEM + 80 mg/ml BSA containing the same radiolabeled compounds as above (80 mg/ml BSA fluid). The same concentration of BSA as rat serum total protein was used; thus, this fluid is artificial rat serum. (4) Testosterone-free rat serum containing the same radiolabeled compounds as above (T-free serum). The four fluids were used in different experiments on the testes and caput epididymides. A fluid was perifused around the seminiferous or epididymal tubules, and interstitial (perifusion) fluid and adjacent intraluminal fluid were collected by micropuncture at one hour after initiation of the sustaining perifusion. Cell-free fluids were aliquoted, and radioactivity of ^{3}H-androgen and ^{14}C-PEG in

the perifusion and intraluminal fluids was determined in all samples. The percentage of peritubular ^{3}H-androgen and ^{14}C-PEG appearing in the intraluminal fluid was determined. Since ^{14}C-PEG is essentially excluded by the blood epididymal barriers, it was included in the perifusion fluid as a marker for contamination of intraluminal fluid by fluid from the extratubular compartments. The proportion of extratubular ^{14}C-PEG appearing in the intraluminal fluid was used to correct the ^{3}H-testosterone data by subtraction.

Measurement of Bound vs Free ^{3}H-Androgen in the Interstitial Fluids in the Testis and Caput Epididymis

One μl of interstitial fluid from the testicular and epididymal peritubular space was obtained at one hour after initiation of sustaining perifusion. Total radioactivity of the fluid sample was determined and then charcoal powder (volume=0.28 mm^{3}; Sigma Chemical Co., St. Louis, MO) was added to the fluid sample. The charcoal was dispersed in the fluid sample by manual shaking, and a micropipette containing the sample was inserted into a rotor head on an IEC model B-20 refrigerator centrifuge. The sample was centrifuged at 5000 rpm for 10 min at 4°C to remove the charcoal containing unbound ^{3}H-androgen. Triplicate aliquots of each supernatant were assayed by scintillation spectrophotometry to determine protein-bound ^{3}H-androgen in the remaining fluid. The radioactivity of protein-bound ^{3}H-androgen was expressed as a percentage of the total radioactivity of the sample fluid.

Data Analysis

Chauvenet's criterion was applied to all the data.[7] The results are presented as mean and SEM. All multiple comparisons were made by the Kruscal Wallis test[8] for nonparametric data, followed by the Wilcoxon rank sum test ($p<0.05$).

RESULTS

^{14}C-PEG concentrations remaining in the testicular peritubular fluids were generally stable in all groups. These values were approximately 85% of those in the original perifusion fluid (Fig. 1). ^{3}H-androgen concentrations remaining in the testicular peritubular fluids in the control group were 14.3±1.9% of those in the original perifusion fluid (Fig. 1). These values were significantly increased in the 8 mg/ml BSA fluid group (41.3±4.4%) and the 80 mg/ml BSA fluid group (68.6±2.2%) ($p<0.02$). A similar result was obtained in the T-free serum group (68.5±3.7%). The residual ^{3}H-androgens in the testicular interstitial fluid increased with increasing albumin concentration in the perifusion fluid.

Intraluminal ^{3}H-androgen concentrations in the seminiferous tubule fluid in the control group were 8.4±2.6% of those in the peritubular fluid (Fig. 2). These values were not significantly decreased in the 8 mg/ml and 80 mg/ml BSA fluid groups nor in the T-free serum group ($p=0.1$), but proluminal movement of ^{3}H-androgen into the seminiferous tubule in both the 80 mg/ml BSA fluid group (0.62±0.29%) and the T-free serum group (0.6±0.18%) was significantly less than that in the 8 mg/ml BSA fluid group (2.3±0.5%; $p<0.01$; Fig. 2). The percentage of bound ^{3}H-androgen in the testicular interstitial fluid in the control group after 1-hr perifusion was 5.2±0.75% (Fig. 3). This value was significantly increased in the 8 mg/ml BSA group (13.7±1.5%) and the 80 mg/ml BSA group (25±0.9%). A similar result was obtained in the T-free serum group (23.2±0.9%).

^{14}C-PEG concentrations remaining in the epididymal interstitial fluids were generally stable in all groups. These values were approximately 80% of those in the original perifusion fluid, and

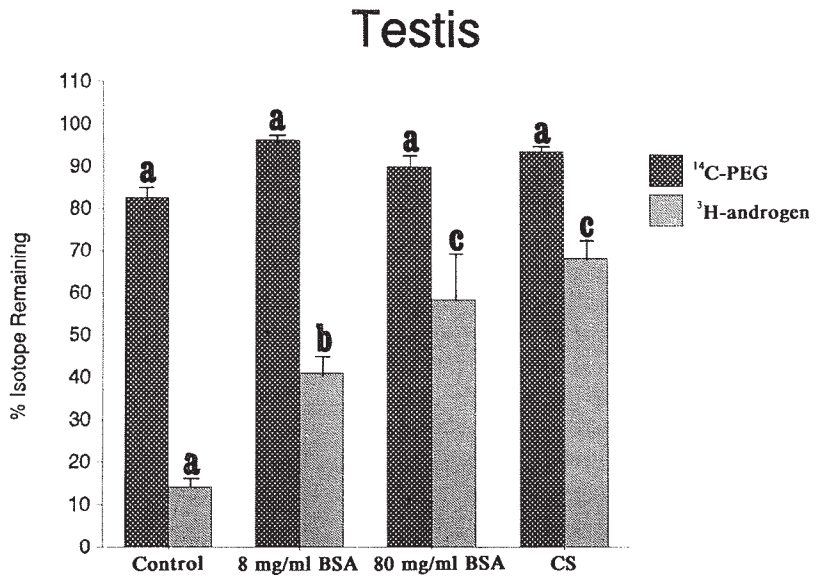

Fig. 1. Percentage of original perifusion fluid ^{14}C-polyethyleneglycol (^{14}C-PEG) or ^{3}H-androgen concentration remaining in the testicular interstitial fluid at one hour after initiation of the sustaining perifusion with four different fluids.
Control: Lissamine-green-dyed Minimum Essential Medium (MEM) Containing 26.7 μCi ^{3}H-testosterone and 1.3 μCi ^{14}C-PEG; 8 mg/ml BSA: MEM + 8 mg/ml Bovine Serum Albumin containing the above radiolabeled compounds; 80 mg/ml BSA: MEM + 80 mg/ml bovine serum albumin containing the same radiolabeled compounds as above; CS: Castrate rat serum containing the same radiolabeled compounds as above. Columns of ^{14}C-PEG sharing same letter superscript are not significantly different ($p=0.09$). Columns of ^{3}H-androgen sharing same numerical superscript are not significantly different.

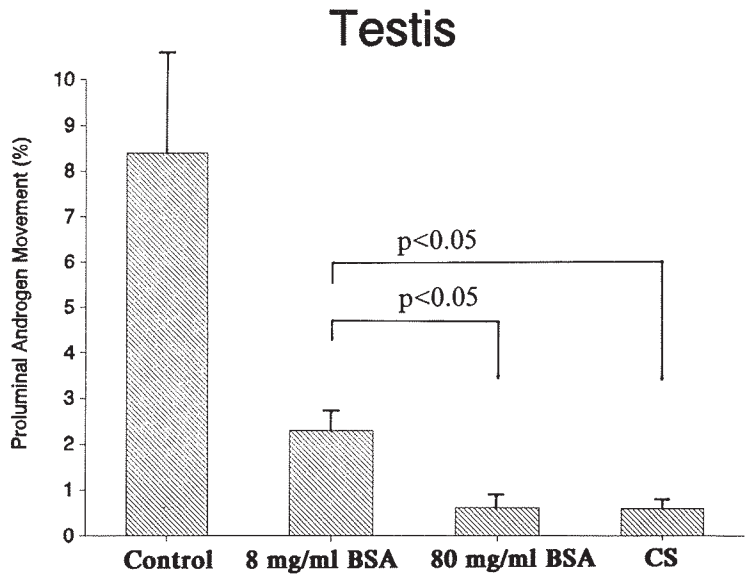

Fig. 2. Proluminal movement of ^{3}H-androgen perifused in the testicular interstitial space at one hour after initiation of the sustaining perifusion with the four different fluids described in Fig. 1. There was no statistical difference between the control group and other groups, but a significant difference between the 8 mg/ml BSA group and 80 mg/ml BSA group or CS group was found ($p<0.01$).

they were similar to those observed in the testis experiment (Figs. 1 and 4). ^{3}H-androgen concentrations remaining around the tubules in the control group were 22.5±3.7% of those in the original perifusion fluid (Fig. 4). These values were significantly increased in the 8 mg/ml BSA fluid group (32±4.4%) and the 80 mg/ml BSA fluid group (58.6±4.1%) ($p<0.02$). A similar result was obtained in the T-free serum group (57.6±6.0%). The residual ^{3}H-androgens in the epididymal interstitial fluid increased with increasing albumin concentration in the perifusion fluid.

Intraluminal ^{3}H-androgen concentrations in the epididymal fluid in the control group were 323.4±73.2% of those in the peritubular fluid (Fig. 5). These values were significantly decreased in the 8 mg/ml and 80 mg/ml BSA fluid groups as well as in the T-free serum group (144.3±20.7%, 70±6.4%, and 75.8±3.8%, respectively; $p<0.05$). Percentage of bound ^{3}H-androgen in the epididymal interstitial fluid in the control group after 1-hr perifusion was 6.4±1.0%, which is similar to the value obtained in the testis experiment (Figs. 3 and 6). This value was significantly increased in the 8 mg/ml BSA group (12.9±0.24%) and the 80 mg/ml BSA group (24±0.9%) ($p<0.05$). A similar result was obtained in the T-free serum group (25.4±3.5%).

DISCUSSION

The present study has shown that intraluminal ^{3}H-androgen concentration in the seminiferous tubule fluid is only 8% of the peritubular ^{3}H-androgen concentration, and this proluminal an-

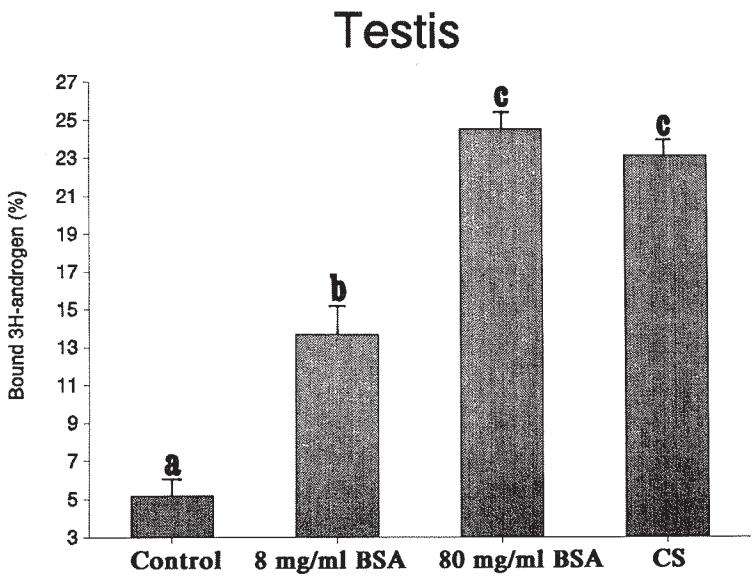

Fig. 3. Percentage of bound ^{3}H-androgen in the testicular interstitial fluid at one hour after initiation of the sustaining perifusion with the four different fluids described in Fig. 1. Columns sharing the same letter superscript are not significantly different ($p=0.21$). Percentage of bound ^{3}H-androgen in the peritubular fluids was significantly increased with increasing albumin concentration in the perifusion fluid ($p<0.01$).

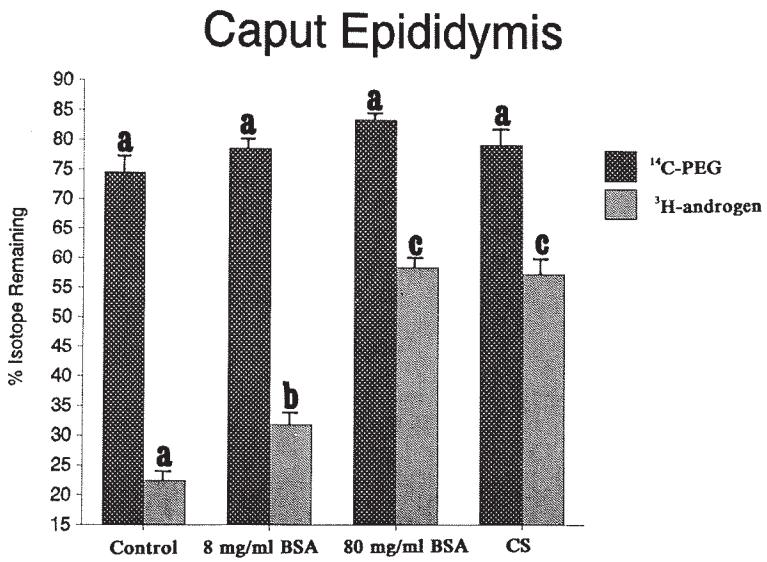

Fig. 4. Percentage of original perifusion fluid ^{14}C-PEG or ^{3}H-androgen concentration remaining in the epididymal interstitial fluid at one hour after initiation of the sustaining perifusion with the four different fluids described in Fig. 1. Columns of ^{14}C-PEG sharing the same letter superscript are not significantly different ($p=0.15$). Columns of ^{3}H-androgen sharing the same numerical superscript are not significantly different.

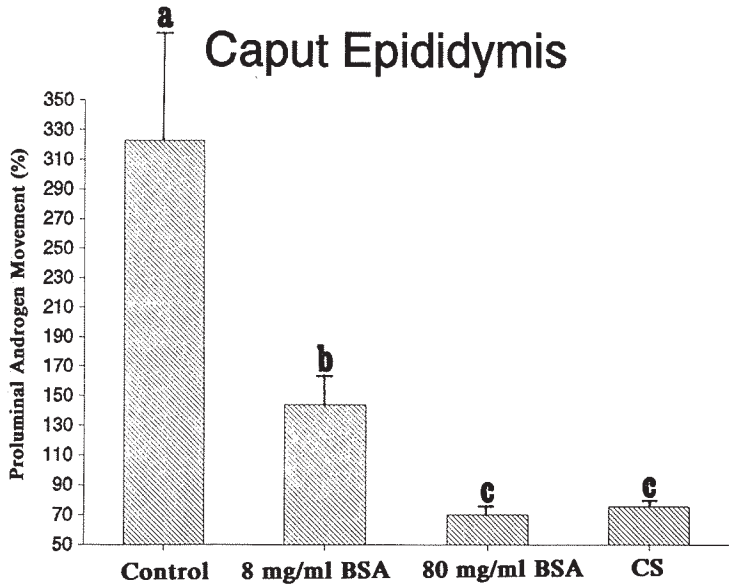

Fig. 5. Proluminal movement of ^{3}H-androgen perifused in the epididymal interstitial space at one hour after initiation of the sustaining perifusion with the four different fluids described in Fig. 1. Columns sharing the same letter superscript are not significantly different. Intraluminal ^{3}H-androgen concentrations in the epididymal fluid were significantly decreased with increasing albumin concentration in the perifusion fluid ($p<0.01$).

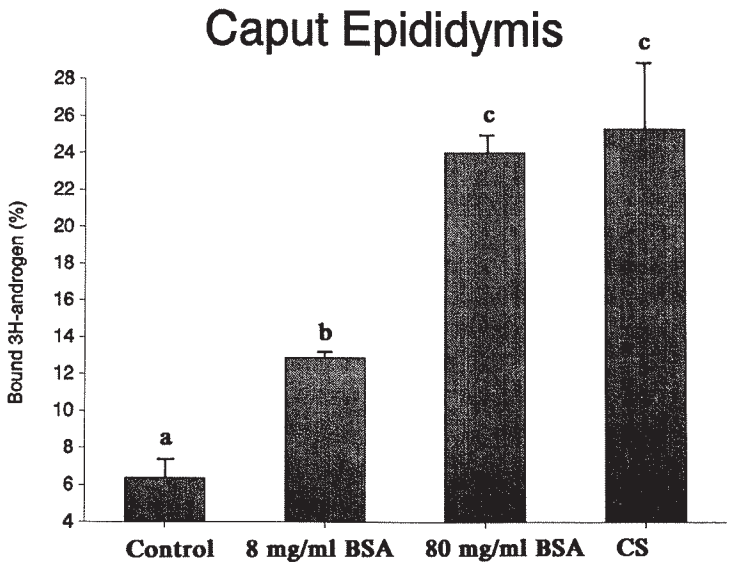

Fig. 6. Percentage of bound ^{3}H-androgen in the epididymal interstitial fluid at one hour after initiation of the sustaining perifusion with the four different fluids described in Fig. 1. Columns sharing the same letter superscript are not significantly different ($p = 0.21$). Percentage of bound ^{3}H-androgen in the peritubular fluids significantly increased with increasing albumin concentration in the perifusion fluid ($p < 0.02$).

drogen movement is not inhibited by the presence of albumin or testosterone-free serum in the perifusion fluid. Total protein and albumin levels in rat testicular interstitial fluid are essentially the same as those found in serum.[6] Concentration of rat serum total protein is 60 to 80 mg/ml. Therefore, perifusion with 80 mg/ml BSA fluid or T-free rat serum around the seminiferous tubules approximates the native condition of the rat testicular interstitial space. When ^{3}H-testosterone was infused into the systemic circulation, one hour after ^{3}H-testosterone infusion intraluminal concentrations of ^{3}H-androgens in the seminiferous tubules were approximately 14% that of blood plasma.[3] When ^{3}H-testosterone was presented directly to the basal aspect of the seminiferous epithelium, intraluminal concentrations of ^{3}H-androgens at one hour after initiation of sustaining perifusion around the seminiferous tubules (movement of ^{3}H-androgen from interstitium to lumen) were 8% to 20% of the interstitial fluid,[3,4] which was essentially similar to the proluminal androgen movement from blood to lumen.

Proluminal movement of ^{3}H-androgen in the testis was significantly decreased with increasing albumin concentration in the perifusion fluid from 8 mg/ml to 80 mg/ml. This result is consistent with the significant increase in ^{3}H-androgen remaining around the tubules and percentage of bound ^{3}H-androgen in the interstitial fluid after one-hour perifusion. Since serum albumin is the main carrier protein for testosterone in the interstitial fluid,[12] it is possible that when albumin concentration in the perifusion fluid is increased, more ^{3}H-androgen binds to interstitial albumin, which retains ^{3}H-androgen and removes some portion of the ^{3}H-androgen pool in the peritubular space available for diffusion into the tubule lumen, and subsequently reduces the proluminal movement of ^{3}H-androgen. Christensen et al. have shown the detailed distribution of serum albumin in the rat testicular space by immunocytochemistry.[9] They observed albumin in

the interstitial tissue, as well as in the space between the boundary layer and the base of the seminiferous epithelium.[9] If perfusion fixation was performed in a manner that flushed most of the albumin from the interstitial space, then this resembled the condition under sustaining perifusion around the tubules, and a layer of albumin remained on the surface of Leydig cells and many macrophages.[9] It is suggested that albumin on the Leydig cell surface may play a role in the mobilization of newly secreted testosterone at the cell surface.[9] Therefore, the question remains of whether or not native albumin localized around Leydig cells during perifusion with fluid containing no BSA can trap diffusible ^{3}H-androgen in the peritubular space and then restrict proluminal movement of ^{3}H-androgen.

Approximately 20% of androgen-binding protein is released into the interstitial compartment, probably from the base of Sertoli cells.[10] Although androgen-binding protein or other androgen-binding molecules in the testicular interstitial space can retain ^{3}H-androgen, it is unclear how much they contribute to the restriction of movement of androgens into the tubule lumen. The percentage of bound ^{3}H-androgen in the testicular interstitial fluid after one-hour perifusion with the control fluid was similar to that in the epididymal interstitial fluid. Based on this result, it can not be explained by differential androgen binding in the interstitial compartment that proluminal androgen movement in the testis is different from that in the caput epididymis where proluminal androgen movement is very high. The reason for restricted transepithelial movement of androgen in the seminiferous tubule is unclear.

The present study has demonstrated that transepithelial movement of ^{3}H-androgen in the caput epididymis is significantly decreased with increasing albumin concentration in the perifusion fluid. Net entry of ^{3}H-androgen into the epididymal tubule was reduced to less than half of the control values by addition of 8 mg/ml BSA to the perifusion fluid. This value was reduced to 70% by addition of 80 mg/ml BSA to the perifusion fluid. These results are consistent with the significant increase in ^{3}H-androgen remaining around the tubules and the percentage of bound ^{3}H-androgen along with the increasing dalbumin concentration in the perifusion fluid. The significant decrease in proluminal ^{3}H-androgen movement by increasing albumin concentration in the perifusion fluid can be accounted for by the significant reduction of free, diffusible ^{3}H-androgen in the interstitial space. There have been no reports of protein concentration in the epididymal interstitial space. However, based on the data for other organs, interstitial protein concentration is approximately one sixteenth of serum.[11] If this is true in the epididymis, significant reduction of proluminal antigrade androgen movement caused by the same concentration of protein in the perifusion fluid as in serum should not happen in the native condition.

Nevertheless, proluminal movement of ^{3}H-androgen across the epididymal tubules was altered in the absence or presence of various degrees of ^{3}H-androgen binding to albumin or other peritubular androgen-binding molecules in the vascular and interstitial compartments, while proluminal movement of ^{3}H-androgen across the seminiferous tubules was not significantly affected by the presence of ^{3}H-androgen binding in the testicular interstitium. Different interstitial concentrations of protein seem to cause different effects of albumin in the perifusion fluid on proluminal movement of ^{3}H-androgen into the tubules of the testis and epididymis.

We have recently demonstrated that proluminal antigrade movement of ^{3}H-androgen in the caput epididymis is completely eliminated by addition of a metabolic inhibitor that blocks ATP production in the metabolic pathway to the perifusion fluid.[14] It was thought to be possible that a metabolic inhibitor interfered with the capillary endothelium and then the interstitial capillary vessels became leakier; thus, more labeled androgen bound to interstitial secreted protein, and unbound androgen in the interstitial space was decreased. Only unbound androgen can enter the epididymal lumen. Therefore, proluminal androgen movement was reduced. The exact mechanism of antigrade androgen movement across the epididymal epithelium, in contrast to the semi-

niferous epithelium, is not known. Nevertheless, it has been demonstrated that intraluminal androgen-binding protein,[5,13] and the presence of a metabolic inhibitor or albumin in the interstitial space have a substantial effect on this unique uphill proluminal androgen movement. These findings make it more problematic to clarify the mechanism of androgen movement specifically across the caput epididymal epithelium.

In conclusion, transepithelial ^{3}H-androgen movement in the testis is not significantly affected by addition of albumin to the perifusion fluid, while proluminal movement of ^{3}H-androgen into the caput epididymal tubules is significantly reduced by increasing albumin concentration in the perifusion fluid. Proluminal movement of ^{3}H-androgen into the seminiferous tubule lumen is restricted[3,4] but not inhibited by competition with unlabeled testosterone,[5] and not significantly affected by the presence of albumin in the perifusion fluid. However, it is possible that increasing androgen binding to protein in the testicular interstitial space may affect proluminal androgen movement. The reason for the low access of androgens to the seminiferous tubule lumen is still unclear.

Antigrade, proluminal movement of ^{3}H-androgen into the caput epididymal tubule lumen is subject to competitive inhibition[5] and significantly decreased by increasing albumin concentration in the perifusion fluid. Differential ^{3}H-androgen binding to peritubular molecules actually alters availability of interstitial ^{3}H-androgen to the intraluminal compartmentalization of androgens in the epididymis. Further experiments will be needed to understand the mechanisms underlying androgen movement across the seminiferous and epididymal epithelia.

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