

The Testicular and Epididymal Luminal Amino Acid Microenvironment in the Rat

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Concentrations of amino acids were measured in arterial and testicular venous blood, and in fluids from the seminiferous tubule, rete testis, and the caput, corpus, and cauda epididymidis. There were no significant differences in the concentrations of any amino acids between arterial and testicular venous blood, whereas there were significant differences between arterial/venous blood and testicular interstitial fluid. The predominant amino acids measured within seminiferous tubule fluid (STF) and rete testis fluid (RTF) were glycine, alanine, glutamate, and glutamine. RTF contained approximately equal concentrations of basic and total amino acids, but 17 times higher acidic amino acids and 1.2 and 1.3 times lower uncharged polar and nonpolar amino acids, respectively, compared to STF. The concentration of total amino acids within caput fluid reached over 50 mmol/L, but then declined to approximately 50% and 0.1% of caput for corpus and cauda, respectively. The predominant amino acids measured within epididymal luminal fluids were glutamate and taurine; glutamate contributed to ~90% of the total amino acids measured in caput fluid. The presence of glutamate and taurine within the epididymal lumen is due primarily to a direct contribution from the epididymal epithelium, as measured using the split-drop stopped-flow microperfusion technique. Several other amino acids within the lumen also originate from the epididymal epithelium. Amino acids contribute approximately 20%, 9%, and 2% of the total osmolality of caput, corpus, and cauda fluid, respectively. Even

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when all ions and organic solutes are measured and compared to the total osmolality of luminal fluid within each epididymal region, there is still a difference of approximately 10, 70, and 35 mOsm/kg water for caput, corpus, and cauda, respectively.

Key words: Microperfusion, glutamate, osmolality, absorption, secretion.

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The epididymal luminal microenvironment is comprised of a number of ions, organic solutes, and macromolecules. In the distal regions of the rat epididymis, more than half of the total osmolality of this fluid milieu is contributed by the organic solutes, whereas in the human ductus deferens, ions are the major contributors (Hinton, 1980). However, when total concentrations of organic solutes and ions within rat epididymal luminal fluid are compared to osmolality, there is a difference of ~100 mOsm/kg water between the two values. This difference, or osmotic deficit, was first recognized by Levine and Marsh (1971). They showed that if just the total number of ions were compared to the total osmolality of epididymal luminal fluid, there was an osmotic deficit of 50

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mmol/L in the seminiferous tubule and more than 250 mmol/L within the cauda epididymidis. These workers postulated that it was secretion of organic acids which made up the deficit. Currently, it seems that carnitine, inositol, and glycerophosphorylcholine contribute a further 100 to 150 mmol/L toward this deficit (Setchell and Hinton, 1981; Robaire and Hermo, 1988). Presumably, the remaining contributors are amino acids, sialic acids, and fatty acids. The current study tested the hypothesis that epididymal luminal amino acids are a major contributor to the total osmolality of epididymal luminal fluid. Experiments also were directed toward understanding the mechanisms by which the epididymal luminal amino acid microenvironment is formed.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (450 to 630 g; Retired Breeders, Hilltop, PA) were used in the study. They were maintained on a 12-hour light:12-hour dark cycle and had free access to food and water.

Collection of Blood Plasma

Carotid arterial blood and testicular venous blood were collected to test the hypothesis that the testicular amino acid arteriovenous differences were zero. In addition, plasma values were needed to determine the amino acid concentration differences between the testicular and epididymal epithelia. Rats were anesthetized with sodium pentobarbital (60 mg/kg), and 1 mL arterial blood was collected via a cannula inserted into the carotid artery. Approximately 100 to 200 μ L testicular venous blood was collected by piercing the testicular vein just as it leaves the surface of the testis; blood was collected into a heparinized tube. Blood was centrifuged at 12,000g for 15 minutes at 4°C in a microcentrifuge. Plasma was mixed with half its volume of deproteinizing agent (1 g/ml 5-sulfosalicylic acid dihydrate, 0.11 g/ml lithium chloride, 60 mg/ml lithium hydroxide monohydrate, 0.98 mg/ml norleucine) and centrifuged at 12,000g for 30 minutes at 4°C. Supernatant was stored at -20°C until analyzed for amino acid content.

Collection of Testicular Interstitial Fluid

Testicular interstitial fluid was collected and analyzed for amino acid content to test the hypothesis that the amino acid composition in interstitial fluid is similar to venous blood. A portion of tunica albuginea was carefully dissected from the underlying seminiferous tubules and blood vessels. Approximately 20 to 30 μ L of testicular interstitial fluid was collected within several minutes from the surface of seminiferous tubules using a glass micropipette. The sample was centrifuged at

12,000g for 15 minutes at 4°C in a microcentrifuge to remove any cell debris. Supernatant was deproteinized as described for blood plasma and then stored at -20°C until analyzed for amino acid content.

Collection of Luminal Fluid from the Testis and Epididymis

To understand the composition of testicular and epididymal amino acid luminal microenvironment, experiments were designed to measure the concentration of amino acids within testicular and epididymal luminal fluids. Animals were anesthetized with an intraperitoneal injection of sodium 5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid (Inactin[®], 160 mg/kg body weight) and prepared for micropuncture of seminiferous tubules and epididymal duct as previously described (Hinton et al, 1979). Seminiferous tubular fluid was collected by micropuncture of tubules, whereas rete testis fluid (RTF) was collected by micropuncturing efferent ducts where they emerge from the testis (Hinton et al, 1979).

From preliminary experiments, it was obvious that conventional micropuncture techniques did not collect sufficient volumes of epididymal luminal fluids for amino acid analysis within a reasonable amount of time: approximately 4 to 5 hours were required to collect 5 to 10 μ L luminal fluid. The length of time needed to collect this volume was considered too long since amino acid analysis of luminal fluid could reflect sperm metabolism while spermatozoa were still in the micropipette and not the true luminal amino acid microenvironment. Hence, a new microperfusion system was developed. With this system, it was possible to collect ~10 μ L luminal fluid within 2 hours. Although 2 hours is not ideal, it was an improvement over the previous time.

For each epididymal region, proximal caput (site 2, Hinton et al, 1979), corpus (sites 4 and 5), and proximal cauda (site 6), a micropipette containing Sudan Black-stained mineral oil was inserted into the duct, and the oil was gently perfused into the lumen at rates of 0.3 to 0.5 μ L/minute, depending upon epididymal region. These perfusion rates were sufficient to cause a slight increase in peristalsis but did not distend the duct. At some distance downstream from the perfusion pipette, a collection pipette was inserted into the duct and luminal contents were collected. In some cases, samples collected from corpus regions were pooled from different animals in order to perform amino acid analysis. To remove spermatozoa, samples were centrifuged at 20,840g for 30 minutes at 4°C in a Sorvall RC-5B centrifuge with a SA 600 rotor. Four to five microliters of supernatant were diluted 1:3 to 1:5, depending upon volume. An equal volume of deproteinizing agent was added. The sample was centrifuged again at 12,000g for 15 minutes at 4°C in a microcentrifuge, and supernatant was stored at -20°C until analyzed for amino acid content.

To calculate what concentration of amino acids would be within the luminal fluid of the caput, corpus, and cauda epididymidis assuming that amino acids were neither metabolized nor absorbed along the duct, the following equation was used: $(I_{RTF} \times F_{RTF})/F_{ER}$, where I_{RTF} = observed concentration of amino acids within RTF,

F_{RTF} = volume of fluid/volume of sperm within RTF which corresponds to 29.3, and F_{ER} = volume of fluid/volume of sperm within each epididymal region which corresponds to 3.85, 1.81 and 1.21 for proximal caput, mid-carpus and proximal cauda, respectively (Hinton et al, 1980).

Measurement of Rate of Secretion of Amino Acids into Epididymal Lumen

To understand the formation of epididymal amino acid luminal microenvironment, it is important to understand the contribution of secretions by the epididymal epithelium to this microenvironment. In this instance, the split-drop stopped-flow technique of Hinton and Hernandez (1985) was used to estimate rate of secretion of amino acids into lumina of tubular segments of the caput, corpus, and cauda epididymidis (sites 2, 4, and 6, respectively; Hinton et al, 1979). An oil droplet previously microinjected into the epididymal duct was split by perfusion with approximately 15 to 20 μ L artificial luminal fluid, composed to resemble the ionic environment, pH, and osmolality of epididymal luminal fluid at each region (Levine and Marsh, 1971; Table 1), at 0.3 to 0.5 μ L/minute. Perfusion was stopped for 60 to 90 minutes and fluid was collected from the duct and analyzed for amino acid content. It was possible to collect approximately 50% of the originally perfused volume within 5 to 10 minutes. The rate of appearance of each amino acid was expressed as pmol/h per mm^3 luminal volume.

Amino Acid Analysis

Amino acid content of each sample was measured using a D-300 ion exchange amino acid analyzer (Squillaro, 1984). In this instance, amino acids within the supernatant were separated on a 0.23×25 cm column containing DC4A cation exchange resin using the Hi-Phi lithium eluent system (Dionex Corporation, Sunnyvale, CA). Amino acids were then postcolumn derivatized with o-Phthaldehyde (Pierce, Rockford, IL; prepared according to manufacturers' directions) and fluorescence was measured using a Gilson fluorometer (360 nm excitation, 455 nm emission). A quantitative report of the concentrations of amino acids within each sample was gener-

Table 1. Composition of artificial luminal fluids

Component	Caput	Corpus	Cauda
NaCl (mmol/L)	100	60	25
KHCO ₃ (mmol/L)	25	40	20
KCl (mmol/L)	—	—	40
Pipes (mmol/L)	20	20	20
Raffinose* (mmol/L)	85	150	150
Osmolality (mOsm/kg water)	320–340	320–340	290–310
pH	6.5	6.7	6.8

* Raffinose was used to make up the osmolality of each epididymal fluid.

ated using the internal standard (norleucine) method (Squillaro, 1984).

Statistical Analysis

Data were analyzed by Chauvenet's criterion (Worthington and Geffner, 1943), t-test, or analysis of variance (ANOVA) followed by Tukey's range test. Mean values were considered significantly different at a 95% level of probability.

To simplify the analysis, comparisons of means were only performed between regions. In addition, to understand the distribution of charged and uncharged amino acids in each region, amino acids were grouped as follows: basic (lys, arg, his), acidic (asp, glu, tau), uncharged polar (asn, gln, ser, thr, tyr), and nonpolar (gly, ala, val, leu, iso, phe, met, trp).

Results

Amino Acid Concentrations within Arterial Blood, Testicular Venous Blood, and Testicular Interstitial Fluid

Concentrations of amino acids within arterial blood, testicular venous blood, and testicular interstitial fluid is shown in Table 2. There were no significant differences in the concentration of each

Table 2. Concentration of amino acids within arterial blood, testicular venous blood, and testicular interstitial fluid

Amino acid	Arterial blood	Testicular venous blood	Testicular interstitial fluid
	$\mu\text{mol/L}$		
Tau	114 \pm 12*	139 \pm 17*†	203 \pm 24†
Asp	52 \pm 3*	54 \pm 4*	13 \pm 4†
Thr	242 \pm 17*	238 \pm 12*	338 \pm 16†
Ser	226 \pm 9*	227 \pm 10*	232 \pm 11†
Asn	86 \pm 5*	88 \pm 2*	117 \pm 11†
Glu	144 \pm 21*	128 \pm 11*	78 \pm 13*
Gln	633 \pm 28*	599 \pm 32*	866 \pm 87†
Gly	279 \pm 24*	293 \pm 19*	324 \pm 4*
Ala	631 \pm 54*	660 \pm 27*	585 \pm 18*
Val	168 \pm 20*	157 \pm 17*	193 \pm 9*
½ Cys	67 \pm 6*	51 \pm 2*†	38 \pm 3†
Met	52 \pm 3*	55 \pm 3*†	67 \pm 3†
Iso	69 \pm 9*	64 \pm 8*	70 \pm 2*
Leu	119 \pm 18*	116 \pm 14*	144 \pm 7*
Tyr	59 \pm 3*	69 \pm 2*	104 \pm 14†
Phe	53 \pm 4*	58 \pm 4*	80 \pm 3†
Trp	156 \pm 19*	168 \pm 22*	26 \pm 1†
Lys	410 \pm 27*	416 \pm 15*	461 \pm 59*
His	83 \pm 5*	76 \pm 5*	87 \pm 5*

Values are expressed as mean \pm SEM, n = 3 to 4 animals. Mean values with different superscripts within each fluid compartment are significantly different (P < 0.05).

amino acid between arterial and venous blood. However, there were some significant differences in the concentration of amino acids between arterial/venous blood and interstitial blood. Amino acids that were significantly higher in concentration in interstitial fluid than in arterial blood included tau, thr, asn, gln, met, tyr, and phe; those lower were asp, $\frac{1}{2}$ cys, and trp. Amino acids that were significantly higher in concentration in interstitial fluid than in venous blood included thr, asn, gln, tyr, and phe; those lower were asp and trp.

Amino Acid Concentrations within the Luminal Fluid of the Seminiferous Tubule, Rete Testis, Caput, Corpus, and Cauda

The concentrations of amino acids measured within the luminal fluids of the seminiferous tubule (STF), rete testis (RTF), caput, corpus, and cauda epididymidis is shown in Table 3. A typical chromatogram of amino acid content of caput fluid is shown in Fig 1. Although there were several amino acids that revealed significant differences in their concentrations between regions, the amino acid showing a high concentration in testicular and epididymal luminal fluids was glutamate (caput: $50,226 \pm 3,683 \mu\text{mol/L}$). The concentration of glutamate then declined significantly to $19,477$

$\pm 3,798 \mu\text{mol/L}$ in the corpus and to $473 \pm 82 \mu\text{mol/L}$ in the cauda ($P < 0.05$). Taurine was found to be greater than 1 mmol/l within luminal fluid of all epididymal regions and reached its highest concentration within the corpus ($6,696 \pm 569 \mu\text{mol/L}$). The majority of the remaining amino acids either declined in their concentration from caput to corpus to cauda or maintained their concentration along the epididymal duct.

The concentrations of measured acidic amino acids exceeded the expected concentration at each epididymal region, whereas concentration of measured amino acids within other groups was less than expected (Fig 2). The concentration of total measured amino acids was lower than expected at each region (Fig 2). From caput to cauda, there was a loss of approximately 0.1, 50, 0.8, 1.5, and 52 mmol/L basic, acidic, uncharged polar, nonpolar, and total amino acids, respectively (Fig 2).

Rate of Secretion of Amino Acids into the Lumina of Different Epididymal Regions

Rate of secretion of amino acids into the lumina of the caput, corpus, and cauda epididymidis is shown in Table 4. The amino acid showing the highest secretion rate into the caput lumen was glutamate ($2,733 \pm 818 \text{ pmol/h per mm}^3$ luminal

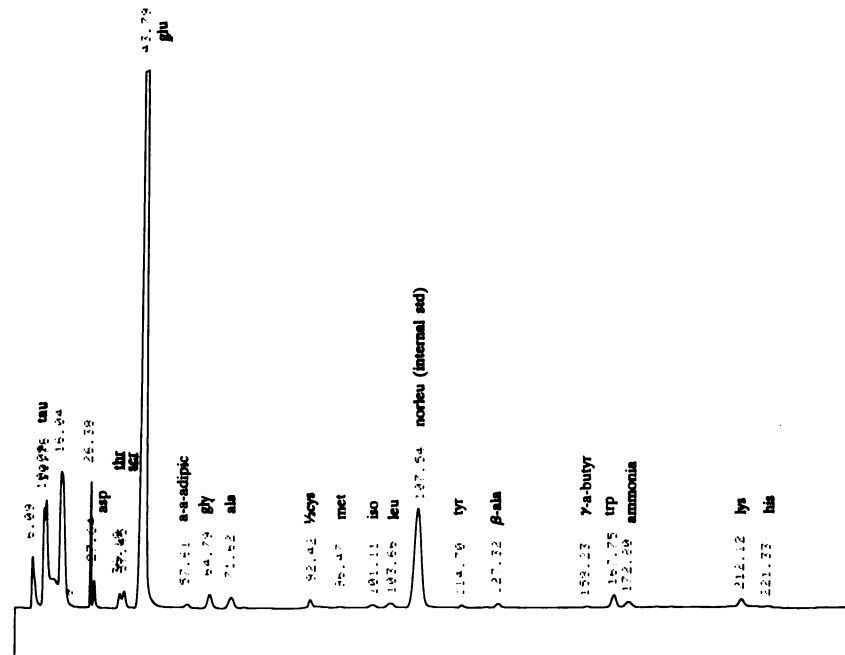
Table 3. Concentration of amino acids within the luminal fluid of the rat seminiferous tubule, rete testis, caput, corpus, and cauda epididymidis

Amino acid	Seminiferous tubule fluid $\mu\text{mol/L}$	Rete testis fluid $\mu\text{mol/L}$	Caput ($\mu\text{mol/L}$)	Corpus ($\mu\text{mol/L}$)	Cauda ($\mu\text{mol/L}$)
Tau	$200 \pm 87\ddagger$	$39 \pm 11\ddagger$	$2120 \pm 74\ddagger$	$6696 \pm 596^*$	$2889 \pm 664\ddagger$
Asp	$205 \pm 48^{*\ddagger}$	nd	$601 \pm 245^*$	$62 \pm 54^{*\ddagger}$	$35 \pm 15\ddagger$
Thr	$299 \pm 98^{*\ddagger}$	$277 \pm 71\ddagger$	$638 \pm 91^*$	$186 \pm 93\ddagger$	$76 \pm 27\ddagger$
Ser	$285 \pm 60^*$	$266 \pm 49^*$	$248 \pm 77^*$	$667 \pm 118^*$	$108 \pm 24^*$
Glu	$917 \pm 48^*$	$33 \pm 16^*$	$50226 \pm 3683\ddagger$	$19477 \pm 3798\ddagger$	$473 \pm 82^*$
Gln	$794 \pm 180^{*\ddagger}$	$1004 \pm 165^*$	nd	nd	$254 \pm 152\ddagger$
Gly	$2447 \pm 246^*$	$3075 \pm 444^*$	$438 \pm 79\ddagger$	$699 \pm 149\ddagger$	$204 \pm 50\ddagger$
Ala	$1038 \pm 165\ddagger$	$1835 \pm 444^*$	$530 \pm 92\ddagger$	$491 \pm 89\ddagger$	$163 \pm 39\ddagger$
Val	$273 \pm 24^{*\ddagger}$	$243 \pm 29^{*\ddagger}$	$277 \pm 88^{*\ddagger}$	$410 \pm 49^*$	$114 \pm 21\ddagger$
$\frac{1}{2}$ Cys	$101 \pm 64^*$	nd	$90 \pm 44^*$	nd	$18 \pm 18^*$
Met	$53 \pm 8^*$	$114 \pm 23^*$	$86 \pm 45^*$	$79 \pm 25^*$	$8 \pm 3^*$
Iso	$36 \pm 13^{*\ddagger}$	nd	$51 \pm 18^{*\ddagger}$	$89 \pm 25^*$	$10 \pm 7\ddagger$
Leu	$146 \pm 21^{*\ddagger}$	$70 \pm 9^{*\ddagger}$	$116 \pm 29^{*\ddagger}$	$194 \pm 65\ddagger$	$41 \pm 13^*$
Tyr	$92 \pm 47^*$	nd	$92 \pm 25^*$	$68 \pm 26^*$	$22 \pm 12^*$
Phe	$92 \pm 46^{*\ddagger}$	nd	$56 \pm 17\ddagger$	$197 \pm 52^*$	$23 \pm 12\ddagger$
Trp	$119 \pm 74\ddagger$	nd	$1336 \pm 340^*$	$400 \pm 41\ddagger$	$630 \pm 130\ddagger$
Lys	$433 \pm 49^{*\ddagger}$	$603 \pm 106^*$	$154 \pm 27\ddagger$	$327 \pm 148^{*\ddagger}$	$108 \pm 30\ddagger$
His	$276 \pm 35^*$	$78 \pm 11\ddagger$	$111 \pm 24\ddagger$	$49 \pm 21\ddagger$	$49 \pm 34\ddagger$

Values are expressed as mean \pm SEM, $n = 5$ to 9 animals. For statistical purposes, all non-detectable values within individual samples were treated as zero. Amino acid mean values with different superscripts within each region are significantly different ($P < 0.05$). Asn was not detected in all samples analyzed.

nd = not detected.

Fig 1—Chromatogram showing the elution profile of amino acids from caput luminal fluid. Sperm-free caput fluid was diluted 1:3, deproteinized as described in materials and methods, and loaded onto a Dionex D-300 component system amino acid analyzer. Amino acids were separated on a DC4A cation exchange resin using the Hi-Phi eluent system. Flow rate was 0.2 ml/min, and temperature at the beginning and end of the run was 41°C and 65°C, respectively. After separation, amino acids were postcolumn derivatized with *o*-phthaldehyde in a 1 minute mixing coil and fluorescence-measured using a Gilson Spectra/Flo fluorometer. Excitation wavelength was 360 nm, and emission wavelength was 455 nm. Numbers located at the top of each peak correspond to the time (minutes) of the run. Tau = taurine; asp = aspartic acid; thr = threonine; ser = serine; glu = glutamate; α -a-adipic = α -amino adipic acid; gly = glycine; ala = alanine; $\frac{1}{2}$ cys = cystine; met = methionine; iso = isoleucine; norleu = norleucine (internal standard); tyr = tyrosine; β -ala = β -alanine; trp = tryptophan; lys = lysine; his = histidine.



volume). Its secretion rate in the caput lumen was not significantly different from that in the corpus (709 ± 264) but was significantly different from that in the cauda lumen (145 ± 50 ; $p < 0.05$). Taurine showed a significant increase in secretion rate from caput to corpus ($P < 0.05$), but then declined in the cauda ($P < 0.05$). Other amino acids that showed a similar pattern of secretion included serine and alanine. The secretion rate of the remaining amino acids either declined along the epididymal duct or was not significantly different from proximal to distal epididymis.

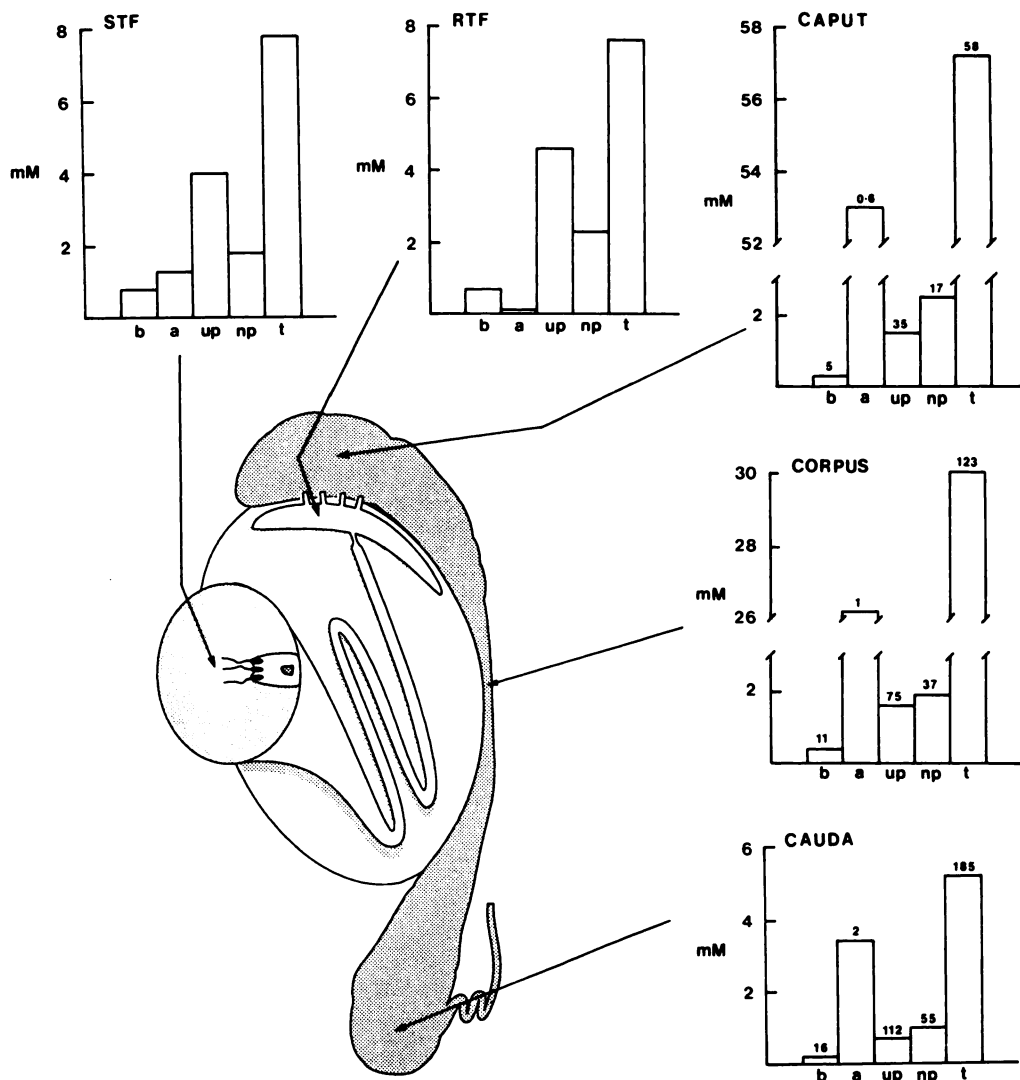
Discussion

The composition of the testicular and epididymal luminal microenvironment is complex. Spermatozoa are bathed in an ever-changing milieu as they progress from the seminiferous tubule to the ductus deferens. Presumably, this fluid milieu is important for their maturation and survival. It is suspected that the microenvironment changes considerably within short distances along the epididymal duct. Currently, however, it is only possible to measure such changes between longer distances, ie, between the caput, corpus, and cauda. Hence, it should be realized that the data presented here only represent the composition and secretion of amino acids within that particular

tubular segment of the caput, corpus, and cauda. This point is particularly important regarding measurements of amino acids within the seminiferous tubule. These measurements represent the net amino acid concentration within an entire seminiferous tubule; the concentration and composition probably varies at discrete stages of the seminiferous epithelium. Despite this limitation, this study has demonstrated that spermatozoa are surrounded by a changing amino acid microenvironment as they progress along the excurrent duct. This study has also shown that the function of the epididymal epithelium is a significant factor in the formation of the luminal amino acid microenvironment.

As STF enters into the rete testis, there are considerable changes in the concentration of ions, organic solutes, and macromolecules, although the concentration of spermatozoa appears to be constant (Setchell et al, 1978). This report has shown that the concentration of several amino acids in RTF is lower than in STF. The decline in the concentration may be due to several factors, including metabolism of amino acids by the spermatozoa as they progress into the rete, uptake by the rete, and possibly metabolism by the rete epithelium as STF enters the rete. It is noteworthy that the rete testis lost almost one 20th of the acidic amino acids generated from the seminiferous tubule, yet gained

Fig 2—Concentration (mmol/L) of basic (b; lys, arg, his), acidic (a; asp, glu, tau), uncharged polar (up; asn, gln, ser, thr, try), nonpolar (np; gly, ala, val, leu, iso, phe, met, trp), and total (t) amino acids within seminiferous tubule fluid (STF), rete testis fluid (RTF), and caput, corpus, and cauda luminal fluids. Numbers on histograms of caput, corpus, and cauda reflect what the concentration of each group of amino acids would be if no amino acids were absorbed with this fluid.



one and one half times the nonpolar amino acids. The total loss of amino acids was quite minimal (approximately 2%).

Testicular fluid enters the initial segment and more than 90% of the water is reabsorbed (Levine and Marsh, 1971; Hinton and Turner, 1988). Hence, the concentration of many compounds that had originated within the testis and enter the epididymis is influenced by water movement. It is important to determine if the measured concentration of any solute is due to direct secretion by the epididymis, to water reabsorption, or to a combination of both. The large gain in concentration of total amino acids observed within the caput epididymidis can be accounted for by water reabsorption as the amino acids moved from the rete testis to the proximal epididymal regions (57.17 mmol/L measured compared to 58.16 mmol/L expected, Fig

2). However, this calculation can be corrected because the major contributor to the total measured amino acid concentration in caput fluid was glutamate, which increased from $33 \pm 16 \mu\text{mol/L}$ in RTF to $50,226 \pm 3,683 \mu\text{mol/L}$ in caput. The presence of this high concentration of glutamate within caput fluid cannot be accounted for by water reabsorption alone, but is the result of either a contribution from the caput epithelium or spermatozoa. The concentration of basic, uncharged polar, and nonpolar amino acids within caput fluid can be attributed to water reabsorption, since the expected concentration was greater than the measured concentration (Fig 2).

The high levels of glutamate found within caput fluid probably originated from the hydrolysis of glutathione by the actions of gamma-glutamyl transpeptidase (GGT; Agrawal and Vanha-Pert-

Table 4. Rate of secretion of amino acids into the lumina of the rat caput, corpus, and cauda epididymidis

Amino acid	Caput (pmol/h/mm ³ luminal volume)	Corpus (pmol/h/mm ³ luminal volume)	Cauda (pmol/h/mm ³ luminal volume)
Tau	152 ± 31*	542 ± 68†	19 ± 19*
Asp	24 ± 5*	39 ± 22*	13 ± 3*
Thr	36 ± 29*	75 ± 31*	55 ± 55*
Ser	22 ± 14*	265 ± 32†	76 ± 9*
Glu	2733 ± 818*	709 ± 264*†	145 ± 50†
Gln	54 ± 53*	85 ± 42*	755 ± 57†
Gly	128 ± 53*	473 ± 171*	107 ± 18*
Ala	37 ± 19*	293 ± 46†	105 ± 15*
Val	63 ± 18*	105 ± 18*†	9 ± 9*
Tyr	171 ± 106*	nd	792 ± 45†
Trp	688 ± 180*	48 ± 26†	168 ± 34†
Lys	49 ± 21*	45 ± 19*	nd

Values are expressed as mean ± SEM, n = 3 to 5 animals. For statistical purposes, all non-detectable samples were treated as zero. Amino acid mean values with different superscripts within each region are significantly different (P < 0.05). Ser, asn, ½cys, met, iso, leu, phe, and his were not detected in all samples analyzed.

nd = not detected.

tula, 1988; Hinton et al, unpublished data). The activity of GGT is highest within the caput region; its activity is almost negligible in the distal epididymal regions (DeLap et al, 1977; Agrawal and Vanha-Perttula, 1988; Hinton et al, unpublished data). This correlates well with *in vivo* studies which showed that the secretion of glutamate was approximately 20 times greater in the caput than in the cauda. Although the term "secreted" is used, it might be more appropriate to use the term appearance to denote that glutamate was derived through the metabolism of a luminal precursor. The exact origin of the glutamate precursor, glutathione, is not known but it is suspected to originate from the testis and via direct secretion into the lumen of the caput. The former suggestion is possible in view of the findings of Shimazaki et al (1976), who showed that following efferent duct ligation, the concentration of caput glutamate declined. However, there was still approximately 30% of caput glutamate remaining following ligation. This data may signify also that efferent duct ligation affected the enzyme GGT.

Taurine was also a major contributor to the total acidic amino acids found within caput fluid. Its appearance cannot be attributed to water reabsorption and therefore must be the result of either direct transport into the lumen from the cell or a precursor. This study has shown that the epididymal epithelium was capable of secreting con-

siderable amounts of taurine into the lumen, particularly within the corpus.

The high concentration of amino acids (particularly glutamate) measured within the caput fluid contributes to approximately 20% of the osmolality of caput fluid (Levine and Marsh, 1971) but only to approximately 9% and 2% of corpus and cauda, respectively. These values are, however, insufficient to make up the remaining osmotic deficit of the epididymal luminal fluids. When all ions and organic solutes known and measured so far are summed and compared to the total osmolality, there still is a deficit of approximately 10, 70, and 35 mOsm/kg water for caput, corpus, and cauda, respectively (Fig 3). The remaining osmotic deficit may be due to the presence of other organic acids, for example, lactic acid.

From caput to cauda, the concentration of total amino acids declined from approximately 60 mmol/L to 5 mmol/L, a loss of ~50mmol/L amino acids, the majority being the loss of glutamate. The loss of measured luminal amino acids from proximal to distal epididymis is an underestimate, since, if water reabsorption is taken into account,

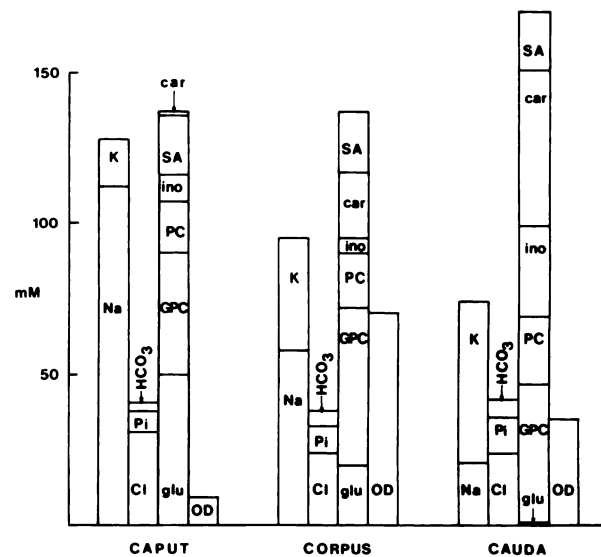


Fig 3—Concentration of ions and organic solutes within luminal fluid of the caput, corpus, and cauda epididymidis. Data for ions are from Levine and Marsh (1971); data for concentration of free sialic acid are from Verawatnapakul and Pholpramol (1988); and data for other organic solutes are from the current and previous studies (see Setchell and Hinton, 1981 for review). Na = sodium; K = potassium; Cl = chloride; P_i = inorganic phosphate; HCO₃ = bicarbonate; glu = glutamate; GPC = glycerophosphorylcholine; PC = phosphorylcholine; SA = free sialic acid; ino = inositol; car = carnitine; OD = osmotic deficit (osmolality minus total number of osmotically active species). Adapted from Levine and Marsh (1971).

the expected concentration of total amino acids within cauda fluid is 185 mmol/L compared to the 5mmol/L that was measured. Presumably, the loss of amino acids was due to absorption since it has been shown that glutamate is readily transported out of the cauda lumen but not out of the caput and corpus lumina (Hinton et al, unpublished data). There are also specific neutral amino acid transport systems located on the apical surface of epididymal cells (Hinton and Hernandez, 1987). The loss of luminal amino acids from caput to cauda could also be due to sperm metabolism, but studies by Setchell et al (1967) and Brooks (1979) suggest that epididymal spermatozoa do not appear to readily use amino acids as a source of metabolic energy. Incorporation of amino acids into the maturing spermatozoa is still a possible explanation for this decline.

The reason for the production of high intraluminal fluid concentrations of different organic solutes is not clear. Although there have been a number of suggestions for the role of each solute in the process of sperm maturation, the role in osmoregulation originally proposed for inositol in the boar by Mann (1954) is a distinct possibility in light of the studies of Yancey et al (1982). Osmolytes such as carnitine, glutamate, inositol, and glycerophosphorylcholine (GPC) have been considered to be nonperturbing solutes, compared to ions considered as perturbing solutes. High concentrations of nonperturbing solutes do not disrupt key macromolecules (ie, enzymes) and cause loss of function as do perturbing solutes. Both inositol and GPC play a crucial role in osmoregulation of renal cells (Nakanishi et al, 1989; Nakanishi and Burg, 1989). In addition to their specific role in sperm maturation, a similar role for these solutes is possible also for both the spermatozoa and the epididymal epithelial cells.

This study has shown that epididymal luminal amino acids originate from several sources—from the testis, by direct transport from epididymal cell into lumen, and from the metabolism of a precursor. It is noteworthy that even though the net concentration of several amino acids declined from caput to cauda, the epididymal epithelium was still secreting those amino acids, ie, aspartate, threonine, glutamate, alanine, tyrosine, into the lumen.

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