Glutamine Synthetase Activity in Rat Epididymis (43772)

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Abstract. **Glutamine synthetase (GS) activity was measured in the caput and cauda regions of rat epididymis. Specific GS activity in the caput was 27-fold higher than that in the cauda. To compare GS activity within the epididymis to that within other tissues, specific and total GS activities were measured in the brain, liver, testes, kidney, and striated muscle. Caput epididymal specific GS activity was from 4- to 38-fold higher than GS activity in any other tissue; caput total GS activity was equal to that in brain. Epididymal GS activity was rapidly and completely inhibited by preincubation with methionine sulfoximine, a known inhibitor of GS. These results suggest that the high concentrations of GS activity in the caput epididymis may have functional significance in maintaining an optimal microenvironment for sperm maturation, perhaps by restoring luminal acid-base balance, removing ammonium and/or glutamate from the lumen, or supplying glutamine for the production of nucleic acids.**

[P.S.E.B.M. 1994, Vol 206]

Glutamine synthetase (GS) is present in a variety of tissues including brain, liver, kidney, spleen, adipose, testes, skeletal muscle, and stomach (1–7). It is a mitochondrial enzyme responsible for the production of glutamine from glutamate, ammonium, ATP, and either magnesium, manganese, or cobalt (4). In brain and liver, GS is thought to be important in ammonium detoxification (8); its role in other tissues is unknown, and its existence in epididymal tissue has not been reported.

The functions of the epididymis in the male reproductive system are thought to include maturation and storage of sperm, but few investigations have been conducted into the metabolic processes within the epididymis that contribute to these functions. A recent study revealed that total luminal amino acid concentration in the proximal or caput epididymis was 60 m*M*, whereas that in the distal or cauda epididymis was 5 m*M* (9). This difference was due primarily to a decrease in glutamate concentration. The existence of glutamate-removing enzymes such as GS in the epididymal epithelium have not been established.

This study measured GS activity in caput and cauda regions of rat epididymis, compared this to activity in other rat organs, and determined the sensitivity of epididymal GS to methionine sulfoximine, a known inhibitor of cerebral GS (10).

Materials and Methods

Animals. Male, Sprague-Dawley rats (Charles River Breeding Co., Wilmington, MA) weighing 275–325 g were used for all experiments. Rats were housed in stainless steel wire mesh cages in an environmentally-controlled room with a 12:12-hr light:dark cycle and had free access to food (Agway rat chow, Syracuse, NY) and water. Rats were sacrificed between 12 PM and 2 PM by ip injection of barbiturate. All procedures were approved by the Animal Care and Use Committee of the University of New Hampshire.

Tissue Preparation. Following euthanasia, six tissues were quickly excised from each rat and rinsed in cold homogenization buffer (Krebs-Ringer bicarbonate buffer containing 0.1% Triton X 100, pH 7.4): brain, liver, testes, kidney, biceps femoris muscle, and epididymis. The epididymis was sectioned into caput and cauda. Each tissue was then quickly blotted, weighed, and homogenized in four volumes of ice-cold homogenization buffer using three to five 10-sec bursts

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Received October 4, 1993. [P.S.E.B.M. 1994, Vol 206]
Accepted March 8, 1994.

0037-9727/94/2064-0360$10.50/0

of a Polytron at full speed. Epididymal tissues were pooled from two rats if tissue weight was less than 0.3 g. Homogenates were filtered through 500 μ nylon mesh and centrifuged at 4°C, 12,000*g* for 15 min. Aliquots of the supernatant to be assayed were further clarified by centrifugation at 4°C, 15,000*g* for 10 min and samples were diluted 10-fold before assay.

Protein content was determined using a modified Lowry method (11) after extraction of fat in 20 volumes of water-saturated ether followed by a 15-min centrifugation at 12,000*g*. Samples were diluted 20-fold before assay.

Glutamine Synthetase Assay. GS was measured spectrophotometrically using the glutamine-γ-glutamyltransferase assay of Rowe *et al.* (12) with minor modifications. Final reagent concentrations were 150 m*M* glutamine, 50 m*M* imidazole buffer (pH 6.8), 1 m*M* magnesium chloride, 100 m*M* hydroxylamine HCl, 37.5 m*M* potassium arsenate, and 0.1 m*M* disodium ADP. The reaction was initiated with 0.05 ml supernatant; tubes were incubated at 37.5°C for 15 min, during which time the reaction was linear. Blanks included all reagents except ADP and potassium arsenate. Ferric chloride-gamma-glutamylhydroxamate complex formation was measured by absorbance at 535 nm in a Bausch-Lomb spectrophotometer and μmoles product formed was calculated using the molar extinction coefficient of 340.

Methionine Sulfoximine Inhibition. Undiluted homogenate (0.1 ml) was preincubated with 10 m*M* ATP, 20 m*M* magnesium chloride, 10 m*M* 2-mercaptoethanol, 150 m*M* potassium chloride, 100 m*M* imidazole buffer (pH 6.8) and either 83.25 m*M* L-methionine sulfoximine or buffer in a final volume of 2 ml at 37°C as described by Ronzio *et al.* (10). At various times, 0.05 ml of the preincubated homogenate was removed and assayed for GS activity as described above.

Statistics. Data were analyzed using Tukey HSD multiple comparison test and *P* value set at 0.05 (SYSTAT: The System for Statistics; SYSTAT, Inc., Evanston, IL, 1988).

Results

Glutamine synthetase specific and total activities spanned a 38- and 494-fold range, respectively, depending upon the tissue source of the enzyme (Table I). The highest specific activity was measured in caput epididymis (9.57 ± 0.96 μmol/min/mg protein), and this was significantly greater than GS activity measured in all other tissues. Specific activity was similar ($P > 0.05$) among testis, kidney and brain tissue samples (range of 2.35 to 2.73 μmol/min/mg protein) and greater than that of muscle. Interestingly, specific GS activity in the caput epididymis was 27-fold greater than that in the cauda.

Total GS activity of caput epididymis, 109 μmol/min/organ, was not statistically different from that of brain, cauda epididymis, testis, and kidney (Table I). It was approximately one-tenth of liver and muscle, which were 1127 and 1068 μmol/min/organ, respectively.

In order to determine if epididymal tissue GS is similar in nature to brain GS, epididymal tissue homogenate was preincubated with L-methionine sulfoximine, an irreversible inhibitor of GS activity in brain. After 10 sec of preincubation, GS activity was reduced to 37% of control; by 1 min, activity was reduced to nearly zero (Fig. 1). A Lineweaver-Burke plot of GS activity as a function of substrate concentration with or without MSO preincubation (Fig. 2) suggests competitive inhibition.

To determine if the GS activity originated from sperm or from epithelial cells of the epididymis, rat sperm were assayed for GS activity. This was done by mincing testes and epididymal tissue in four volumes of buffer, filtering through nylon mesh, and centrifuging the filtrate at 15,000*g* for 15 min. The pellet containing sperm was resuspended in buffer, homogenized, centrifuged for 15 min, and the supernatant was assayed. No GS activity was detected.

Discussion

This study demonstrates that of six tissues in the rat known to contain glutamine synthetase activity,

Table I. Glutamine Synthetase Activity in Various Rat Tissues[a]

Tissue	Specific activity (μmol/min/mg protein)	Total activity[c] (μmol/min/organ)
Caput epididymis[b]	9.57 ± 0.96[1]	108.72 ± 20.01[1]
Cauda epididymis[b]	0.35 ± 0.24[4,5]	2.28 ± 0.74[1]
Testis	2.40 ± 0.74[2,3]	289.67 ± 97.52[1,2]
Kidney	2.35 ± 0.75[2,3]	306.86 ± 25.33[1]
Brain	2.73 ± 0.69[2]	108.68 ± 29.27[1]
Liver	1.47 ± 0.43[3,4]	1126.55 ± 495.57[3]
Muscle	0.25 ± 0.20[5]	1068.30 ± 865.24[2,3]

[a] Values are mean ± SD, $n = 7$, unless otherwise indicated. Values within a column with different superscripts are significantly different, $P < 0.05$.
[b] $n = 4$.
[c] Total activity calculations were based on organ weights calculated for a Sprague-Dawley rat weighing 328 g (34, 35).

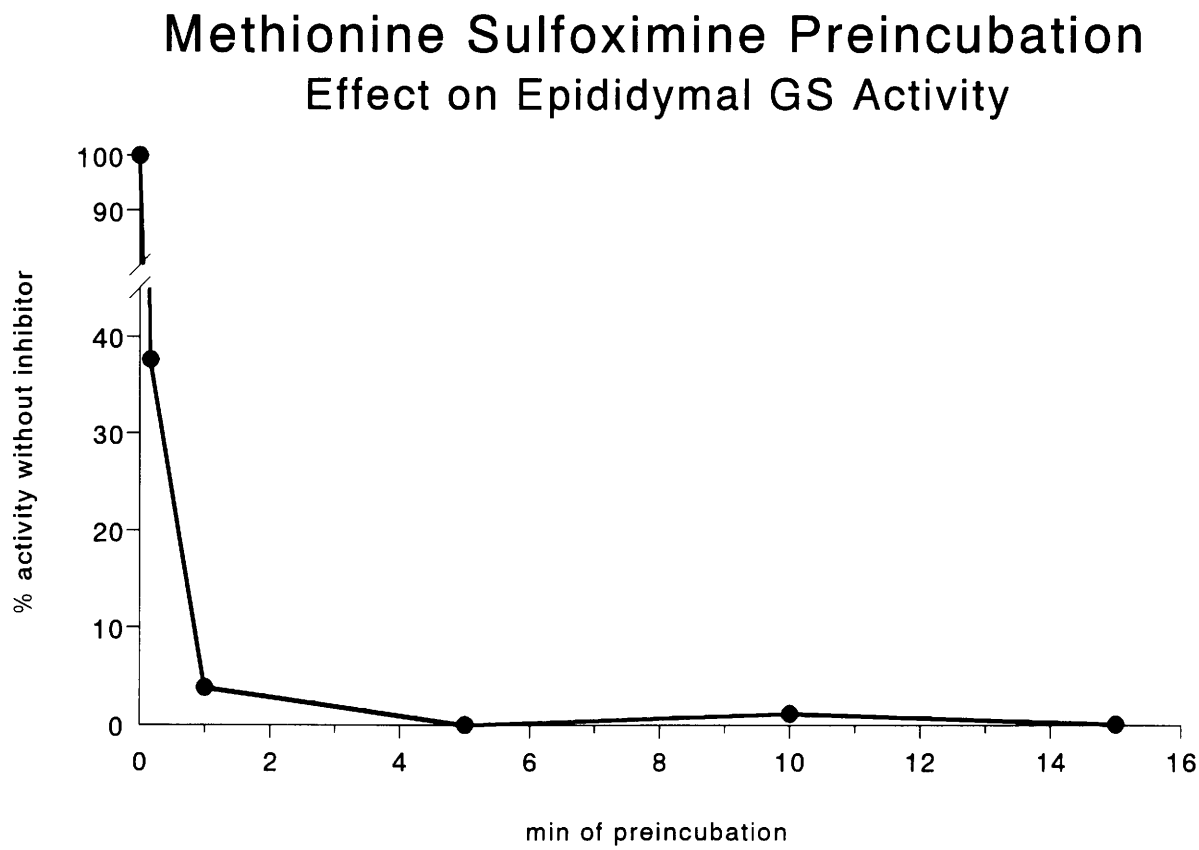

Figure 1. Epididymal tissue homogenate was preincubated with 10 m*M* ATP, 20 m*M* $MGCl_2$, 10 m*M* 2-mercaptoethanol, 150 m*M* KCl, 100 m*M* imidazole buffer, and either buffer or 83.25 m*M* L-methionine sulfoximine at pH 6.8 and 37°C for up to 15 min (final volume = 2 ml). Aliquots were removed at 10 sec, 1 min, 5 min, 10 min, and 15 min, and assayed for GS activity using the glutamine-γ-glutamyltransferase assay of Rowe *et al.* (12).

the highest specific activity is contained in a tissue never reportedly assayed for this enzyme—the caput epididymis. Brain and liver, heretofore considered to be richest in specific GS activity, have 29% and 15% of the activity present in epididymis, respectively. The swift and sharp inhibition of epididymal GS activity by L-methionine sulfoximine, similar to that observed by Ronzio *et al.* for purified sheep brain GS (10), suggests the epididymal and brain isoforms of GS may have similar catalytic natures. This involves the ability of brain GS to form an enzyme-bound γ-glutamyl phosphate intermediate, leading to the phosphorylation of methionine sulfoximine which, in turn, tightly binds to GS and irreversibly inhibits it.

The dramatic drop in specific activity from caput to cauda regions of the epididymis could reflect an important role for GS in this tissue. Immature spermatozoa emerge from the seminiferous tubules of the testis and are exposed to an ever-changing milieu of fluids as they migrate through the excurrent duct of the rete testis on their way to the most distal end of the epididymis. The luminal fluid that bathes spermatozoa as they travel through the epididymis contains a variety of ions, organic solutes, and amino acids which are constantly being absorbed and secreted by the epididymal epithelium (13). Studies show that association and dissociation of certain proteins from sperm cell membranes also occurs (14–21). The metabolic regulation of this complex microenvironment, and the biochemical signaling that exists between the testis, the epididymis, and the surface of the spermatozoa, has not been fully elucidated.

Studies have shown that glutamate exists in high concentration in the caput region of the epididymis (9, 22). Some investigators attribute this to the reabsorption of testicular fluid as it leaves the rete testis and enters the caput region of the epididymis (23). As water is reabsorbed, the impermeable acidic amino acid glutamate would become more concentrated. However, a recent micropuncture study revealed that water reabsorption alone could not account for the high concentration of glutamate found in the lumen of the caput epididymis (9), and the author suggests that either the caput epithelium or spermatozoa are responsible for generating high levels of glutamate.

The precursor for glutamate is unknown, but one candidate is glutathione secreted by the testis or the epididymis into the epididymal lumen (9). γ-Glutamyl transpeptidase (GGT), which hydrolyzes this tripeptide to glutamate, cysteine, and glycine, has very high

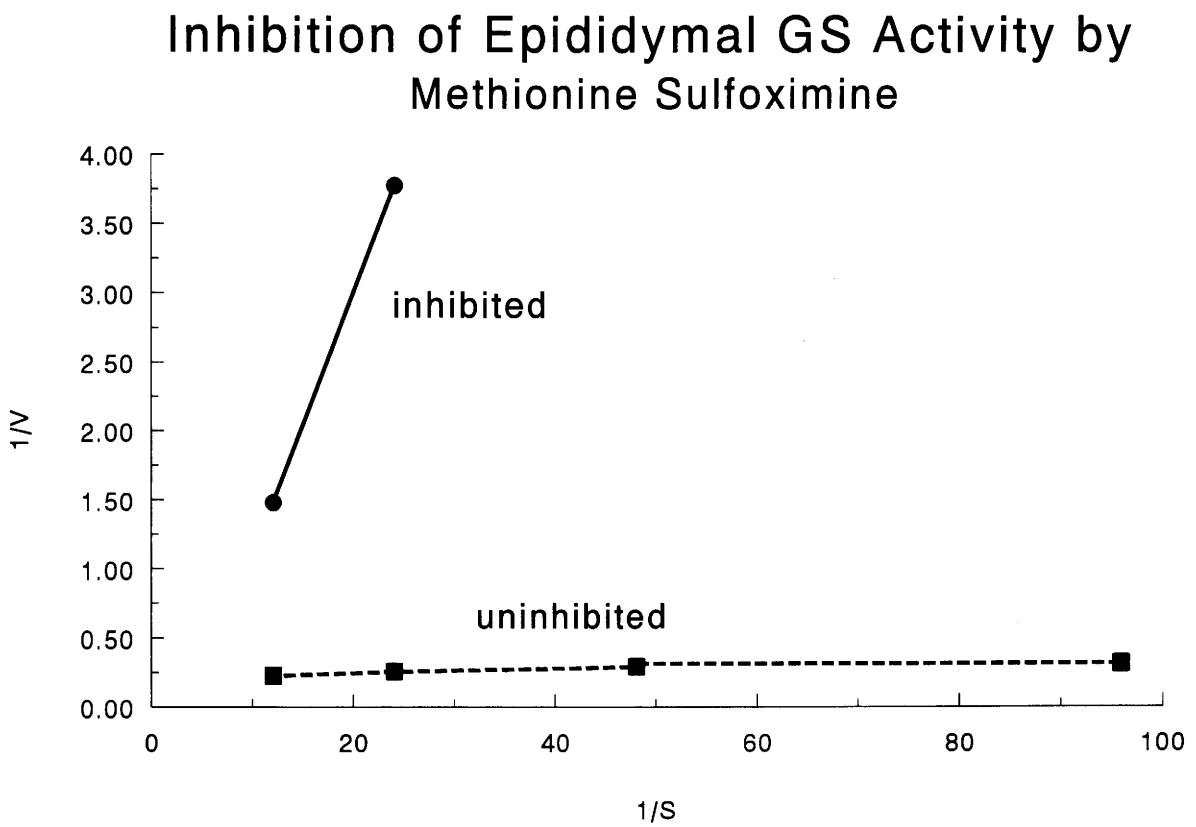

Figure 2. Epididymal tissue homogenate was preincubated with either buffer (uninhibited) or 83.25 m*M* L-methionine sulfoximine (inhibited) for 15 min, as described in Figure 1, then assayed for GS activity at up to four concentrations of substrate. Units for 1/s and 1/v are $(M)^{-1}$ and $(\mu mol/min/mg\ protein)^{-1}$, respectively.

activity in the caput region but negligible activity in the cauda (24, 25). The results of the present study suggest that the glutamate may be removed by the high activity of GS in the caput. Transport of glutamate into the epithelial cells of the epididymis may be via a neutral amino acid transport system on the apical surface of the cell (26).

The luminal pH of the epididymis changes from one section to another. While water, sodium, potassium, and chloride are removed from the caput lumen, hydrogen ions, lactic acid, and glutamate are secreted or formed therein. This results in an acidic caput lumen (pH 6.48) which becomes increasingly more alkaline in the cauda (pH 7.5) (27). Entrance and dissociation of weak organic acids with pHs lower than the seminal plasma's pH would suggest an environment in which GS is necessary for acid-base homeostasis.

Glutamate is one of the amino acids necessary for the immobilization and storage of spermatozoa in the epididymis (22, 28). Glutamate constitutes the highest concentration of the free amino acids, making up 20% of all intraluminal osmolytes (29, 30). As a nonperturbing solute, glutamate could be essential for osmoregulation due to its compatibility with macromolecular structures (9, 31). Therefore, removal of glutamate by conversion to glutamine may allow for enhanced sperm mobility as the sperm mature.

The fact that glutamine is not detected in the epididymal lumen until reaching the caudal section (9) indicates that the glutamine produced is either being utilized by spermatozoa or epithelial cells, or is removed by the blood stream. However, spermatozoa do not use glutamine as a significant source of energy (28, 32). Therefore, it is more probable that glutamine is being used for a separate function such as removal of glutamate or synthesis of purine and pyrimidine bases. Glutamine and GS activity have been implicated in cell differentiation: *in vitro,* glutamine added to media enhances differentiation of male chick embryonal gonads and initiates conversion of gonocytes into pachytene spermatocytes (33), while *in vivo,* GS activity correlates with differentiation of embryonic chick retina (32). Whether or not glutamine serves a similar function in the epididymis is unclear.

In summary, this study compared GS activity in a variety of male rat tissues. The exceedingly high specific GS activity in the caput section of the epididymis, 4- to 38-fold higher than any other tissue measured, suggests a vital role for this enzyme in male rat reproductive physiology. Although the significance of GS activity can only be revealed through further study, it can be hypothesized that this high GS activity may be responsible for enhancing spermatozoan development by restoring proper acid-base balance, removing glu-

tamate from the epididymal lumen, or providing a precursor for nucleic acid synthesis.

This work was supported as Scientific Contribution Number 1841 from the New Hampshire Agricultural Experiment Station.

1. Dierks-Ventling C, Cone AL, Bessman SP. The attachment of glutamine synthetase to brain membranes. Biochem Med **13**:213–223, 1975.
2. Iqbal K, Ottaway JH. Glutamine synthetase in muscle and kidney. Biochem J **119**:145–156, 1970.
3. Krebs HA. Metabolism of amino acids. IV. The synthesis of glutamine, from glutamic acid and ammonia, and the enzymic hydrolysis of glutamine in animal tissues. Biochem J **29**:1951, 1935.
4. Lund P. A radiochemical assay for glutamine synthetase and activity of the enzyme in rat tissues. Comp Biochem Physiol **31**:205–210, 1969.
5. Miller RE. Adipose tissue and skeletal muscle glutamine synthetase in normal and diabetic rats. Diabetes **24**:416P, 1975.
6. Pequin L, Vellas F, Bouche G. La glutamine synthetase chez la carpe (Cyprinus carpio). Arch Sci Physiol **23**:469–480, 1969.
7. Wu C. Glutamine synthetase. A comparative study of its distribution in animals and its inhibition by DL-allo-d-hydroxylysine. Comp Biochem Physiol **8**:335–351, 1963.
8. Cooper A. Glutamine synthetase. In: Kvamme E, Ed. Glutamine and Glutamate in Mammals. Boca Raton, FL: CRC Press, Vol **1**:pp11–19, 1988.
9. Hinton BT. The testicular and epididymal luminal amino acid microenvironment in the rat. J Androl **11**:498–505, 1990.
10. Ronzio R, Rowe WB, Meister A. Studies on the mechanism of inhibition of glutamine synthetase by methionine sulfoximine. Biochemistry **8**:1066–1075, 1968.
11. Markwell MAK, Haas SM, Tolbert NE, and Bieber LL. Protein determination in membrane and lipoprotein samples: manual and automated procedures. In: Berger SL, Kimmel AR, Eds. Methods in Enzymology (1st ed). New York: Academic Press, Vol **72**:pp296–303, 1981.
12. Rowe WB, Ronzio R, Wellner VP, Meister A. Glutamine synthetase. In: Tabor H, Tabor CW, Eds. Methods in Enzymology. New York: Academic Press, Vol **17A**:pp900–910, 1970.
13. Waites GMH, Setchell BP. Physiology of the testis, epididymis, and scrotum. In: McLaren A, Ed. Advances in Reproductive Physiology. New York: Academic Press, p31, 1969.
14. Turner TT, Plesums JL, Cabot CL. Luminal fluid proteins of the male rat reproductive tract. Biol Reprod **21**:883–890, 1979.
15. Olson GE, Hinton BT. Regional differences in luminal fluid polypeptides of the rat testis and epididymis revealed by two-dimensional gel electrophoresis. J Androl **6**:20–34, 1985.
16. Vernon RB, Muller CH, Herr JC, Feuchter FA, Eddy EM. Epididymal secretion of a mouse sperm surface component recognized by a monoclonal antibody. Biol Reprod **26**:523–535, 1982.
17. Voglmayr JK, Sawyer RF, Dacheux JL. Glycoproteins-A variable factor in surface transformation of ram sperm during epididymal transit. Biol Reprod **33**:165–176, 1985.
18. Cornwall GA, Vreeburg JT, Holland MK, Orgebin-Crist MC. Interactions of labeled epididymal secretory proteins with spermatozoa after injection of ^{35}S-methionine in the mouse. Biol Reprod **43**:121–129, 1990.
19. Orgebin-Crist MC, Jahad N. The maturation of rabbit epididymal spermatozoa in organ culture: Inhibition by anti-androgens and inhibitors of ribonucleic acid and protein synthesis. Endocrinology **103**:46–53, 1979.
20. Fournier-Delpech S, Courot M, Dubois MP. Decreased fertility and motility of spermatozoa from rats immunized with prealbumin epididymal specific glycoprotein. J Androl **6**:246–250, 1985.
21. Moore HDM, Smith CA, Hartman TD. The role of the epididymal epithelium in the development of mammalian sperm-fertilizing capacity *in vivo* and *in vitro*. Ann NY Acad Sci **513**:195–203, 1987.
22. Mann T. The Biochemistry of Semen and of the Male Reproductive Tract. London: Barnes & Noble, p181, 1964.
23. Crabo B, Gustafsson B. Distribution of sodium and potassium and its relation to sperm concentration in the epididymal plasma of the bull. J Reprod Fertil **7**:337–345, 1964.
24. DeLap LW, Tate SS, Meister A. Gamma-glutamyl transpeptidase and related enzyme activities in the reproductive system of the male rat. Life Sci **20**:673–680, 1977.
25. Agrawal YP, Vanha-Perttula T. Gamma-glutamyl transpeptidase, glutathione, and L-glutamic acid in the rat epididymis during postnatal development. Biol Reprod **38**:996–1000, 1988.
26. Hinton BT, Hernandez H. Neutral amino acid absorption by the rat epididymis. Biol Reprod **37**:288–292, 1987.
27. Levine N, Marsh DJ. Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. J Physiol **213**:557–570, 1971.
28. Setchell BP, Hinks NT, Voglmayr JK, Scott TW. Amino acids in ram testicular fluid and semen and their metabolism by spermatozoa. Biochem J **105**:1061–1065, 1967.
29. Turner TT, Giles RD. A sperm motility inhibiting factor in the rat epididymis. Am J Physiol **242**:R199–R203, 1982.
30. Usselman MC, Cone RA. Rat sperm are mechanically immobilized in the cauda epididymis by "immobilin," a high molecular weight glycoprotein. Biol Reprod **29**:1241–1253, 1983.
31. Yancey P, Clark M, Hand S, Bowlus R, Somero G. Living with water stress: Evolution of osmolyte systems. Science **217**:1214–1222, 1982.
32. Brooks DE. Biochemical environment of sperm maturation. In: Fawcett DW, Bedford JM, Eds. The Spermatozoon. Baltimore: Urban & Schwarzenberg, pp23–24, 1979.
33. Steinberger A, Steinberger E. Stimulatory effect of vitamins and glutamine on the differentiation of germ cells in rat testes organ culture grown in chemically defined media. Exp Cell Res **44**:429–435, 1966.
34. Melby EC Jr., Altman NH, Eds. Handbook of Laboratory Animal Science. Boca Raton, FL: CRC Press, Vol **3**, 1976.
35. Peachey LD, Adrian RH, Geiger SR, Eds. Handbook of Physiology. Bethesda, MD: American Physiological Society, Sec **10**, 1983.