Zinc Deficiency in Adult Rats Reduces the Relative Abundance of Testis-Specific Angiotensin-Converting Enzyme mRNA^{1,2,3}

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ABSTRACT Zinc deficiency results in reduced testicular angiotensin-converting enzyme (ACE) activity and reduced amounts of ACE protein in the testes of young rats. In the present study, we examined the effect of zinc deficiency on the relative abundance of testicular ACE mRNA and its relationship to ACE activity over time. Forty-five male rats at 7 wk of age were placed on one of three feeding regimens: 1) a diet adequate in zinc, 2) a diet deficient in zinc and 3) a diet adequate in zinc that was fed in an amount equal to that consumed by a paired mate fed the zinc-deficient diet. Rats were killed after 3, 5 and 7 wk. Rats fed the zinc-deficient diet had significantly lower ($P < 0.01$) body weight gain and testis weight at each week sampled than the other groups. They also showed compromised zinc status as evidenced by significantly lower ($P < 0.01$) serum and testis zinc concentrations. At each period, rats fed the zinc-deficient diet had significantly lower ($P < 0.01$) testicular ACE activity than rats fed either of the zinc-adequate diets. Coinciding with low ACE activity, there was a lower ($P < 0.01$) relative abundance of ACE mRNA in the group for the zinc-deficient diet than in either of the zinc-adequate groups. The results suggest that much of the low ACE activity in the testes of rats in the latter stages of zinc deficiency is attributable to a reduction in ACE gene transcription. However, an effect of the deficiency on ACE mRNA turnover is not ruled out.


KEY WORDS: zinc • angiotensin-converting enzyme • testes • mRNA • rats

Zinc deficiency in males of many species, including humans, causes an impairment of sexual development (Millar et al. 1958 and 1960; Sandstead et al. 1967). Previously, this impairment was shown to manifest itself primarily in young animals and consisted of hypogonadism that resulted in arrested development of secondary sex characteristics (Mason et al. 1982). More recent investigations found that adult animals are affected similarly to young animals in that germ cell maturation is suppressed and the testes begin to atrophy when the animals are fed zinc-deficient diets for up to 5 wk (Reeves and Stallard 1995). The exact role that zinc plays in male sexual maturation is unknown, but it may be related partly to the enzymes that require zinc as a catalyst. A zinc-metalloenzyme that is closely related to maturation of the testes, and to its function remains unresolved.

Angiotensin-converting enzyme (ACE, EC 3.4.15.1) is a zinc-metalloenzyme that is important in sexual development and fertility and that its activity is dependent upon an adequate supply of dietary zinc, its function remains unresolved.

In an ongoing effort to determine the association between zinc status and ACE activity, we showed that a reduced amount of ACE protein in zinc-deficient rat testes may be the cause of low ACE activity (Reeves and Stallard 1995). Although these studies suggest that testicular ACE is important in sexual development and fertility and that its activity is dependent upon an adequate supply of dietary zinc, its function remains unresolved.

MATERIALS AND METHODS

The Animal Use Committee of the USDA, ARS, Grand Forks Human Nutrition Research Center approved this study, and the study was performed according to the guidelines of the National Institutes of Health on the experimental use of laboratory animals (NRC 1985).

REFERENCES


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5 Abbreviations used: ACE, angiotensin-converting enzyme; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SDS, sodium dodecyl sulfate; SSC, sodium chloride-sodium citrate; +Zn, zinc-adequate diet; −Zn, zinc-deficient diet; +ZnP, zinc-adequate diet paired fed.
Animals and diet. At wk 7 of age, 45 male Sprague-Dawley rats (Sasco, Madison, WI) were divided into three groups of 15 rats each. One group was fed a basal diet similar to AIN-93G (Reeves et al. 1993) but pellets with the following changes: egg white was dried. The pellets were casein, extra biotin (2 mg/kg diet) was added, the mineral mix did not contain a source of zinc, and the amount of phosphorus was changed so that the finished diet provided 3 g phosphorus/kg diet when the mineral mix was used at 35 g/kg diet. This diet was designated -Zn. Another group was fed the basal diet supplemented with 35 mg zinc/kg diet (+Zn). Each rat in the third group was fed daily an amount of the +Zn diet equal to that consumed the previous day by its mate in the -Zn group; this diet was designated +ZnPF.

At wk 3 and 5, five rats were selected at random from each diet group and anesthetized with sodium pentobarbital (50 mg/100 g body wt). The remaining rats in each group were treated similarly at wk 7. The abdomen was opened and blood was collected from the dorsal aorta into Monovette tubes (Sarstedt, Newton, NC); the blood was allowed to clot at 4°C for 1 h. Serum was separated by centrifugation and frozen at -20°C until analyzed for zinc content. Testes were removed and immediately frozen in liquid nitrogen and then stored at -80°C until used for RNA isolation. Livers were removed, blotted and stored at -20°C until analyzed for zinc content.

Chemicals and reagents. Unless otherwise noted, chemicals were purchased from Sigma Chemical (St. Louis, MO). The 50× Denhardt’s reagent contained 10 g of Ficoll (type 400, Pharmacia Biotech, Uppsala, Sweden), 10 g of polyvinylpyrrolidone and 10 g of bovine serum albumin (fraction V)/L. The 20× SSC contained 3 mol NaCl and 0.3 mol sodium citrate/L.

Total RNA isolation. All glassware and instruments were properly cleaned by standard procedures (Sambrook et al. 1989). Tissue (0.2 g) was removed from a decapitated frozen testis and then homogenized in a glass/glass tissue grinder containing 1.0 mL of buffer (6 mol guanidine thiocyanate, 40 mmol sodium citrate and 90 mmol 2-mercaptoethanol/L). The homogenate was mixed with 100 μL of 2 mol sodium acetate/L and 1.2 mL of phenol-chloroform (1:1 ratio) for 15 min and then centrifuged at 10,000 × g for 20 min at 4°C. The upper aqueous phase was transferred to a fresh tube and 45 mL of 2 mol sodium acetate/L was added followed by 700 μL of cold 100% ethanol. The tube was set aside at -80°C overnight before centrifugation at 13,000 × g for 30 min; the supernatant was decanted and the pellet washed 6 times with 100% ethanol and then dried. The pellet was suspended in TE buffer and the RNA quantitated spectrophotometrically. Both quantity and quality of the RNA sample were verified by gel electrophoresis and staining with ethidium bromide. The RNA samples were stored at -80°C.

Northern analysis of RNA. The isolated testes testes (10 μg) was denatured and separated on a 1% agarose gel containing 2.2 mol formaldehyde/L and transferred to a nylon membrane (Quantum Yield, Promega, Madison, WI) by capillary blotting (Sambrook et al. 1989). Three gels were run, one for each week sampled. Every gel contained lanes representing each dietary treatment as well as a size marker. Blots were prehybridized for 4 h at 42°C in 10 mL of hybridization buffer (5× Denhardt’s reagent, 40% formamide, 10% dextran sulfate, 1 mol NaCl and 50 mmol Tris-HCl/L, pH 8, 0.1% SDS, and 100 mg denatured salmon sperm DNA/L) (Bernstein et al. 1988).

Testicular ACE mRNA was detected by using the ACE.5 probe provided by Kenneth Bernstein (Emory University, Atlanta, GA) and Albert HI (Bernstein et al. 1989). The ACE.5 cDNA was cloned into phagemid Bluescript (Stratagene, La Jolla, CA) and used to transform bacterial cells (Epiciuran Coli XLI-Blue competent cells; Stratagene). The cDNA was separated from the vector by agarose gel electrophoresis after digestion with EcoRI. A control cDNA probe, human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (Clontech, Palo Alto, CA), was used to verify equal RNA loading per lane and to determine the specificity of dietary treatment effects. Both probes were labeled with [α-32P]dCTP (Amersham, Arlington Heights, IL) by random hexamer primer labeling (RadiPrime Labeling System, Gibco BRL, Gaithersburg, MD). Blots were hybridized sequentially, first with GAPDH cDNA at 48°C for 16 h followed by washing at 65°C with the following conditions: 1) 2× SSC, 15 min; 2) 2× SSC, 0.1% SDS, 30 min; 3) 0.1× SSC, 10 min. Then blots were hybridized with ACE.5 cDNA at 42°C for 16 h and washed as follows: 1) 6× SSC, 0.1% SDS at room temperature for 15 min; 2) 0.2× SSC at 62°C for 15 min. Blots were then exposed to autoradiography film (Kodak X-OMAT AR) at -80°C with intensifying screens.

To account for any differences in loading and efficiency of transfer, the abundance of the 2.7-kb ACE mRNA transcript was compared with the abundance of GAPDH mRNA in the same lane of each gel. This was done by densitometric analysis of the bands on the autoradiographs (CIS200 Scanning Densitometer and GS 367W Electrophoresis Data System, version 3.01, Hoefer Scientific Instruments, San Francisco, CA). The heights of the peaks for each band were used for comparison, and a ratio of ACE mRNA to GAPDH mRNA was generated for each lane. Means of four or five lanes from each group on one autoradiograph were calculated and compared statistically.

Total DNA. Total testicular DNA was determined fluorometrically by the method of Downs and Willfinger (1983). Tissue was homogenized in a mixture containing 1 mol NH4OH and 20 g Triton X-100/L. One milliliter of homogenate was incubated with 100 ng of bisbenzimideazol (Hoechst 33258) and the fluorescence read at 350 nm excitation and 455 nm emission.

Other analyses. Angiotensin-converting enzyme activity was measured fluorometrically by detecting His-Leu released from hirudin-histidyl-leucine by the method of Santos et al. (1985) as modified by our laboratory (Reeves and Stallard 1995). Activity was expressed in the katal unit (kat), which corresponds to moles per second. Protein concentrations in testes preparations were determined by using the bichenichonic acid method (Sigma Chemical).

Zinc concentrations of serum, liver and testes were determined by atomic absorption spectrometry. Serum (50 μL) was diluted with 1.0 mL of deionized water, and then 500 μL of 5-sulfoacetic acid dihydrate (0.5 mol/L; Aldrich Chemical, Milwaukee, WI) was added. Samples were then incubated for 48 h at room temperature and centrifuged, and then the supernatant was analyzed for zinc by atomic absorption spectrometry. Tissue samples were lyophilized to constant weight and dry ashed at 450°C. The ash was reconstituted in 5 mL of 1.0 mol HCl/L and then analyzed for zinc by AAS.

Statistical analysis. The data were analyzed by two-way ANOVA (version 4, Crunch Software Corporation, Oakland, CA). Significant differences between group means were determined by a step-down multiple stage F test (REGWF) (Einot and Gabriel 1975, Ryan 1960, Welsch 1977). For two variables, testes ACE activity and ACE mRNA relative density, we could not assume that the data were sampled from a Gaussian population; therefore, we use the Kruskal-Wallis nonparametric ANOVA, and the t statistic for pairwise comparisons between means (Kruskal and Wallis 1952). Data are expressed as means ± SEM.

RESULTS

At each period, rats in the -Zn group had significantly lower (P < 0.01) daily weight gain than rats in either of the zinc-adequate groups (Table 1). Daily gain in body weight also was less (P < 0.01) in the +ZnPF group than in the +Zn group. In rats allowed to eat naturally (groups -Zn and +Zn), weight gain declined over time. In the +ZnPF group, however, the amount gained per day declined between wk 3 and 5 and then stabilized between wk 5 and 7.

Table 1

<table>
<thead>
<tr>
<th>Period</th>
<th>Controls</th>
<th>Zn</th>
<th>ZnPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>wk 3</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>wk 5</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>wk 7</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Indicators of zinc status were lowered in rats fed the -Zn diet. As early as wk 3, serum zinc concentrations were signific-
Zinc deficiency affects the body weight gain, testis weight and testis weight:body weight ratio of rats.\textsuperscript{1,2}

<table>
<thead>
<tr>
<th></th>
<th>Body weight gain</th>
<th>Testis weight</th>
<th>Testis weight:body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/d</td>
<td>g</td>
<td>g/100 g body wt</td>
</tr>
<tr>
<td>Week 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Zn</td>
<td>6.7 ± 0.3\textsuperscript{a}</td>
<td>1.65 ± 0.04</td>
<td>0.543 ± 0.02</td>
</tr>
<tr>
<td>+ZnPF</td>
<td>2.6 ± 0.3\textsuperscript{b}</td>
<td>1.55 ± 0.05</td>
<td>0.713 ± 0.03</td>
</tr>
<tr>
<td>-Zn</td>
<td>1.5 ± 0.4\textsuperscript{c}</td>
<td>1.38 ± 0.14</td>
<td>0.709 ± 0.08</td>
</tr>
<tr>
<td>Week 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Zn</td>
<td>5.5 ± 0.4\textsuperscript{a}</td>
<td>1.66 ± 0.16\textsuperscript{a}</td>
<td>0.470 ± 0.05\textsuperscript{b}</td>
</tr>
<tr>
<td>+ZnPF</td>
<td>1.6 ± 0.2\textsuperscript{b}</td>
<td>1.39 ± 0.05\textsuperscript{a}</td>
<td>0.630 ± 0.03\textsuperscript{a}</td>
</tr>
<tr>
<td>-Zn</td>
<td>0.8 ± 0.1\textsuperscript{c}</td>
<td>0.72 ± 0.06\textsuperscript{b}</td>
<td>0.380 ± 0.03\textsuperscript{b}</td>
</tr>
<tr>
<td>Week 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Zn</td>
<td>4.8 ± 0.1\textsuperscript{a}</td>
<td>1.83 ± 0.12\textsuperscript{a}</td>
<td>0.451 ± 0.02\textsuperscript{b}</td>
</tr>
<tr>
<td>+ZnPF</td>
<td>1.8 ± 0.1\textsuperscript{b}</td>
<td>1.66 ± 0.06\textsuperscript{a}</td>
<td>0.669 ± 0.02\textsuperscript{a}</td>
</tr>
<tr>
<td>-Zn</td>
<td>0.6 ± 0.1\textsuperscript{c}</td>
<td>0.75 ± 0.16\textsuperscript{b}</td>
<td>0.402 ± 0.08\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Values are means ± SEM of five rats. Different superscripts within weeks for each variable denote significant differences among treatments, with \( P < 0.001 \) for body weight and testis weight and \( P < 0.01 \) for testis:body weight ratio.

\textsuperscript{2} The +Zn rats were allowed to eat the control diet naturally; +ZnPF rats were allowed to eat only as much food as their paired mates in group −Zn; −Zn rats were allowed to eat the low zinc diet naturally.

TABLE 2

Zinc deficiency affects the concentration of zinc in serum, liver, and testis of rats without affecting the amount of testicular DNA.\textsuperscript{1,2}

<table>
<thead>
<tr>
<th></th>
<th>Serum zinc</th>
<th>Liver zinc</th>
<th>Testis zinc</th>
<th>Testis DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu \text{mol/L} )</td>
<td>( \mu \text{mol/kg} )</td>
<td>mg/kg</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Zn</td>
<td>23.1 ± 0.7\textsuperscript{a}</td>
<td>380 ± 10</td>
<td>347 ± 6\textsuperscript{a}</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>+ZnPF</td>
<td>18.2 ± 0.9\textsuperscript{b}</td>
<td>400 ± 20</td>
<td>335 ± 17\textsuperscript{a}</td>
<td>6.0 ± 0.7</td>
</tr>
<tr>
<td>−Zn</td>
<td>4.9 ± 0.5\textsuperscript{c}</td>
<td>360 ± 19</td>
<td>231 ± 32\textsuperscript{b}</td>
<td>6.1 ± 0.6</td>
</tr>
<tr>
<td>Week 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Zn</td>
<td>22.3 ± 1.7\textsuperscript{a}</td>
<td>351 ± 18\textsuperscript{b}</td>
<td>346 ± 11\textsuperscript{a}</td>
<td>4.9 ± 0.6</td>
</tr>
<tr>
<td>+ZnPF</td>
<td>14.1 ± 0.6\textsuperscript{b}</td>
<td>435 ± 9\textsuperscript{a}</td>
<td>370 ± 9\textsuperscript{a}</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>−Zn</td>
<td>4.4 ± 0.7\textsuperscript{c}</td>
<td>330 ± 16\textsuperscript{b}</td>
<td>250 ± 27\textsuperscript{b}</td>
<td>4.6 ± 0.7</td>
</tr>
<tr>
<td>Week 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Zn</td>
<td>21.4 ± 0.9\textsuperscript{a}</td>
<td>386 ± 16\textsuperscript{b}</td>
<td>344 ± 8\textsuperscript{a}</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td>+ZnPF</td>
<td>16.5 ± 0.4\textsuperscript{b}</td>
<td>440 ± 11\textsuperscript{a}</td>
<td>340 ± 14\textsuperscript{a}</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>−Zn</td>
<td>5.3 ± 0.4\textsuperscript{c}</td>
<td>347 ± 10\textsuperscript{c}</td>
<td>205 ± 27\textsuperscript{b}</td>
<td>3.6 ± 0.5</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Values are means ± SEM of five rats. Different superscripts within weeks for each variable denote significant differences among treatments with \( P = 0.01 \). Although there was no significant difference among treatments for testis DNA, there was a significant difference (\( P < 0.02 \)) among treatment weeks where wk 3 values were higher than values at wk 5 or wk 7.

\textsuperscript{2} The +Zn rats were allowed to eat the control diet naturally; +ZnPF rats were only allowed to eat as much food as their paired mates in group −Zn; −Zn rats were allowed to eat the low zinc diet naturally.
in four out of five samples were not detectable at wk 5, and three out of five were not detectable at wk 7. At wk 3, ACE bands in all samples were detectable. At each week, the intensity of the control probe was approximately the same in all groups. A representative sample of an autoradiograph from wk 5 is shown in Figure 2.

A statistical analysis of the ACE:GAPDH mRNA ratios was difficult to perform because many of the values in the -Zn group for wk 5 and 7 were zero. However, because of the large variation, we performed the Kruskal-Wallis rank means test, analyzing each week separately. The ACE:GAPDH mRNA ratios during all three periods were significantly ($P < 0.01$) lower in the zinc-deficient rats than in either of the control groups (Fig. 3). Ratios for the +Zn and +ZnPF groups were not different. Because of the nature of the assay, values between weeks could not be compared.

**DISCUSSION**

Angiotensin-converting enzyme is a zinc-metalloenzyme that consists of two isozymes. One is the somatic type and is found in vascular endothelium and kidney. This isozyme is thought to function primarily in the regulation of blood pressure by cleaving angiotensin I to form the vasoconstrictor, angiotensin II (Erdos and Skidgel 1987). It also cleaves the vasodilator bradykinin. This isozyme has a molecular mass of approximately 160 kDa and contains 2 mol of zinc per mole of enzyme protein.

The other isozyme is found only in the testes, has a molecular mass of approximately 95 kDa, and contains only 1 mol of zinc per mole of enzyme (Ehlers and Riordan 1991). The testicular ACE proteins in mice (Bernstein et al. 1989), humans (Ehlers et al. 1989) and (presumably) rats are unique in that they are produced from the exons identical to those used to assemble the carboxyl terminal half of somatic ACE.

Previously we showed that zinc deficiency in rats before puberty caused a reduction in the activity of testicular ACE (Reeves and O’Dell 1988). Although we also demonstrated that zinc deficiency reduced the activity of somatic ACE in serum (Reeves and O’Dell 1985), it seemed to depend on the type of substrate used in the assay, and it required a sufficient supply of zinc in the assay medium. In addition, we could not find a negative effect of the deficiency on lung ACE (Reeves and O’Dell 1988), which seemed to suggest that testicular ACE was unique in its response to zinc deficiency. However, we have since shown that ACE activity in the intestinal mucosa of zinc-deficient young male rats is about 40% less than that in zinc-adequate rats (not published), suggesting that indeed somatic ACE can be affected by zinc deficiency.

Because testicular ACE begins to be expressed only at puberty (Strittmatter and Snyder 1984, Strittmatter et al. 1985), we initially thought that the absence of a sufficient supply of dietary zinc during this period was inhibiting the general development of the testes. However, more recently we showed that zinc deficiency also causes a reduction in testicular ACE activity in adult rats (Reeves and Stallard 1995). In addition, we demonstrated that the reduction in activity was apparently caused in part by a reduction in sex hormone levels. However, because of the nature of the assay, values in different gels could not be compared.

FIGURE 2 Northern analysis of angiotensin-converting enzyme (ACE) mRNA from the testes of zinc-adequate (+Zn), paired fed (+ZnPF) and zinc-deficient (-Zn) adult rats. At 3, 5, and 7 wk, 10 μg of testicular RNA was isolated and separated on denaturing gels (one for each week sampled). RNA was transferred to nylon membranes and hybridized with two [α-32P]dCTP-labeled probes, ACE.5 and glutaraldehyde-3-phosphate dehydrogenase. Only examples from wk 5 are shown in this autoradiograph. Testicular ACE mRNA gave a signal at 2.7 kb and the control probe at 1.4 kb. Although all lanes gave near equal intensity for the control probe, the intensity of the ACE.5 probe in lanes containing RNA from -Zn rats was barely detectable.

**FIGURE 3** Zinc deficiency affects the relative abundance of angiotensin-converting enzyme (ACE) mRNA in the testes of adult rats. Rats were placed on one of three feeding regimens: 1) a diet adequate in zinc (+Zn), 2) a diet deficient in zinc (−Zn), and 3) a diet adequate in zinc that was paired-fed in an amount equal to that consumed by a mate fed the −Zn diet (+ZnPF). At 3, 5 and 7 wk, 10 μg of testicular RNA was isolated and separated on denaturing gels (one for each week sampled). RNA was transferred to nylon membranes and hybridized with two [α-32P]dCTP-labeled probes, ACE.5 and glutaraldehyde-3-phosphate dehydrogenase (GAPDH). Following autoradiography, differences in band intensities were determined by densitometric analysis. Peak heights of each probe were compared within lanes. Bars represent the mean ± SEM of four or five rats. Different letters within weeks denote significant differences by the Kruskal-Wallis rank means test.
specific ACE mRNA was examined. The results clearly show that zinc deficiency in adult male rats will eventually lead to a reduction in ACE mRNA. In turn, this apparently leads to lower ACE protein, which then causes the reduced activity of ACE as observed in zinc-deficient rats. We are interpreting our results to suggest that ACE mRNA transcription is reduced by zinc deficiency. Because we did not measure mRNA turnover, however, the possibility of increased ACE mRNA degradation by zinc deficiency is not ruled out.

Whether reduced germinal cell maturation during zinc deficiency is directly related to the maintenance of ACE protein and enzyme activity or whether the deficiency is causing a general effect on spermatid maturation is still to be determined. We previously showed that the numbers of germ cells and sperm are reduced in the testes of zinc-deficient rats, but those cells that remain have lower ACE activity than cells from zinc-adequate rats (Reeves and Stallard 1995). Sibony et al. (1994) determined the step-wise expression of germinal ACE mRNA and ACE protein in testes of normal rats and mice as the spermatids matured through various stages. By using in situ hybridization with a testis-specific ACE cDNA probe, they found that ACE mRNA was first expressed in stages four to seven, with maximal expression at stages eight to 12. Expression declined through the remaining stages. Immunolocalization of ACE protein showed a similar progression; however, ACE protein remained elevated throughout the latter stages of maturation. Sibony et al. (1994) concluded that ACE is produced exclusively in haploid germ cells of these species. Because zinc is involved in so many aspects of metabolism, it is possible that spermatid development is arrested in the early stages of zinc deficiency and the spermatids never reach the stage where ACE mRNA is normally expressed.

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LITERATURE CITED


