Zinc Deficiency Affects the Activity and Protein Concentration of Angiotensin-Converting Enzyme in Rat Testes (43608)

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Abstract. **Zinc (Zn) deficiency causes hypogonadism in a number of different species. Previous work has shown that Zn deficiency reduces the activity of angiotensin-converting enzyme (ACE), a Zn-dependent enzyme, in the testes of prepubertal rats. These studies were designed to determine whether this effect was caused by a change in the concentration of ACE protein. Thirty-five male rats at 26 days of age were divided into three groups. One group was fed *ad libitum* a Zn-adequate diet (40 mg/kg); another group was fed a similar diet, but deficient in Zn (<1.0 mg/kg); a third group was pair-fed to the deficient group. After 4 weeks on these regimens, all rats in the *ad libitum*-fed group and half of the rats in each of the deficient and pair-fed groups were sacrificed, and tissues were collected for analysis. The remaining animals in the Zn-deficient and pair-fed groups were fed a Zn-adequate diet *ad libitum* for another 2 weeks, then sacrificed. With the use of an enzyme-linked immunosorbent assay for testicular ACE protein, the effect of these treatments on the concentration of ACE protein in testes was determined. After 4 weeks, ACE activity in testes of the Zn-deficient rats was reduced by 74% compared to that in the *ad libitum*-fed controls. This was accompanied by a 64% reduction in the amount of ACE protein in the testes. There was not a significant effect of pair-feeding. Refeeding Zn-deficient rats a Zn-adequate diet for 2 weeks restored ACE protein concentrations and ACE activity to values not significantly different from those in pair-fed controls. Soluble ACE, but not particulate ACE, of the epididymis was significantly reduced by Zn deficiency. Because the ACE activity of testes has been found primarily in the germinal cells, and soluble ACE in the epididymis is derived from the testes, these findings suggest that the effects of Zn deficiency on testicular and epididymal ACE is caused by an impairment of spermatid development.**

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Zinc (Zn) deficiency in young males of a number of species, including humans, results in a failure of sexual development (1–3). The primary manifestation is hypogonadism, which results in diminished secondary sex characteristics. Depending on the severity of the deficiency, the effects may or may not be reversed by supplementing the diet with Zn (3–5).

The exact role that Zn plays in this process is unknown, but could be related to specific enzymic reactions requiring Zn. It has been shown that the testes and epididymides of the adult rat contain high activity of angiotensin-converting enzyme (ACE; EC 3.4.15.1), a Zn-metalloenzyme (6). ACE is not expressed until the beginning of puberty and then increases greatly as these organs mature (7). This suggests that the enzyme is important for sexual development, but its function is still unknown.

We showed recently that the activity of this enzyme in testis and epididymis is affected by Zn deficiency (8–10). When prepubescent rats were fed a Zn-deficient diet for 4 weeks, ACE activity was depressed as much as 65% compared to that in Zn-adequate controls. While the cause of this phenomenon is unknown, there is the possibility that low activity results from the failure of ACE protein to be expressed. To determine if this is the cause, we developed an enzyme-linked immunosor-

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bent assay (ELISA) to measure ACE protein in rat testis. With this assay, we determined the effects of Zn deficiency on the concentration of ACE protein in the testis during the period of maturation.

Preliminary studies in our laboratory suggest that the age of the rat is an important determinant of a Zn deficiency effect on testicular ACE activity. Therefore, we also determined age-related effects of Zn deficiency on the expression of ACE activity in both the testis and epididymis.

Materials and Methods

Design. The data will be reported as two separate studies. In the first study, 35 male rats, 26 days old (Sasco, Lincoln, NE),[3] were randomly divided into three groups. One group of seven rats was fed *ad libitum* a semipurified diet adequate in Zn (40 mg/kg diet; +ZnAL). This diet is described in detail in Ref. 10. Another group of 14 rats was fed a similar diet, but without added Zn (<1 mg/kg; −ZnAL). A third group of 14 rats was fed the Zn-adequate diet, but the amount of diet each rat received was limited to that consumed on the previous day by a weight-paired mate in the −ZnAL group. This group was designated +ZnPF. After 4 weeks on these regimens, all rats in the +ZnAL group and seven rats from each of the other two groups were sacrificed by exsanguination. The remaining seven rats in groups −ZnAL and +ZnPF were fed the Zn-adequate diet *ad libitum* for an additional 14 days.

The second study was similar to the first, except that two different age groups of rats were used (22 and 30 days). This study was carried through the 4-week Zn depletion phase only.

The rats were anesthetized with pentobarbital sodium (50 mg/kg body wt, ip), and blood was collected from the abdominal aorta into Monovette tubes (Sarstedt, Newton, NC). The blood was allowed to clot at room temperature (23 ± 1°C) for 1 hr and centrifuged at 4°C. Serum was collected for Zn analysis. Both testes and one epididymis were removed, weighed, then immediately frozen. The other epididymis was weighed and finely minced in 1 ml of 146 mmol/liter of NaCl and allowed to sit at room temperature for 30 min. Released sperm were counted by using a hemacytometer.

ACE Activity Assay. Part of one testis and one whole epididymis were individually homogenized (1 part tissue to 4 parts buffer) at 4°C in a buffer containing 0.4 *M* NaCl and 50 m*M* HEPES, pH 7.4. The homogenization was carried out for 1 min with a Tissumizer equipped with an SDT-182EN probe (Tekmar, Cincinnati, OH). Samples were centrifuged at 38,000*g* for 40 min at 4°C. The supernatants were collected and frozen. This fraction contained the salt-soluble ACE (11). The pellets were resuspended in a volume of buffer equal to that of the original supernatant. To solubilize membrane-bound ACE, this buffer also contained 0.5% Triton X-100 detergent (low peroxide grade, Pierce, Rockford, IL) and 10 μmol of Zn/liter. The pellets were incubated at 4°C for 2 hr, then centrifuged. The supernatants were collected and frozen. This fraction contained Triton X-soluble ACE. ACE activities in these fractions were determined as described previously (12, 13).

ACE Protein Determination. An ELISA was developed to determine the ACE protein concentration in rat testes. Briefly, ACE protein was isolated and purified by affinity chromatography. Lisinopril affinity gel was either generously supplied by Dr. Bart Holmquist, Harvard Medical School, or made in our laboratory by the methods of Bull *et al.* (14) and Hooper and Turner (15), as modified by Schullek and Wilson (16). Antibodies to the purified protein were produced in rabbits and used to coat polystyrene tubes. ACE protein was conjugated to alkaline phosphatase (ACE-AP) to be used as the detector. The assay is based on the competition between ACE in the sample and the ACE-AP conjugate for binding sites on the antibody-coated tubes. ACE-AP was added in an amount that would bind approximately 50% of the sites on the coated tubes. Aliquots of testicular supernatant solubilized with Triton X-100 or known quantities of ACE protein standard were added, and the tubes were incubated at 4°C for 18 hr. The tubes were washed with binding buffer, and the amount of ACE-AP bound was determined by the addition of AP substrate, *p*-nitrophenylphosphate. This reaction produced a yellow chromaphore, which was read at 400 nm. A standard curve of the ratio of absorbance of each standard to the absorbance without standard versus known amounts of ACE protein was linearized with a log-logit plot, and the amount of ACE in the unknown samples was calculated.

The limit of detection of ACE protein by this ELISA procedure is approximately 5 ng. The antibody to testicular ACE protein will cross-react with somatic ACE. However, because somatic ACE is present in very small amounts in the testis, it should not interfere with our determinations. Detailed procedures for the ELISA may be obtained from us by request (17).

Other Assays and Statistical Analyses. γ-Glutamyl transferase activity was determined in the Triton X-solubilized particulate portion of the testis by modifying a procedure from Sigma Chemical Co. (Kit 545-A, St. Louis, MO). For mineral analyses, tissues were weighed to the nearest 0.1 g and lyophilized. The dried samples were weighed and then ashed in a muffle furnace at 450°C for 48 hr. The ash was dissolved in 1 mol/liter of HCl, and Zn was determined by atomic absorption spectrometry. Quality control was assured

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

by simultaneous assay of certified standards of bovine liver samples from the National Bureau of Standards (Lot 1577a). Zn concentrations in all standards were within ±5% of the certified values.

Data were analyzed by the one-way analysis of variance procedures. We used the statistical software package from Crunch Software Corp. (Oakland, CA). Hartley's F_{max} test (18) for homogeneity of variances was run with each analysis of variance. When the test was significant, that is variances were not homogeneous, the data were transformed to achieve or approach homogeneity and analyzed. Data from the transformations were presented along with the original data where appropriate. Significant differences between group means were determined by a step-down multiple stage F test (REGWF) (19–21).

Results

Study 1. Young male rats were fed Zn-deficient or control diets for 4 weeks, then the deficient rats were refed Zn-adequate diets for 2 weeks longer. Table I shows the effects of these regimens on body weight gain and tissue weights. As expected, Zn deficiency significantly reduced weight gain during the depletion period; this was partly caused by a voluntary reduction in food intake. Although the pair-fed group consumed the same amount of food as the Zn-deficient group, they had greater weight gains than the deficient group. This suggests that the difference in weight gain between the two groups was caused specifically by the lack of Zn. Refeeding Zn-adequate diets *ad libitum* to both −ZnAL and +ZnPF groups significantly ($P < 0.001$) increased weight gain. Total testicular weight was significantly affected by Zn deficiency and pair-feeding. However, when weight was expressed as a function of body weight, the difference was lost. Epididymal weights followed the same trends as those of the testis (Table I). However, when expressed as a function of body weight, Zn deficiency significantly reduced epididymal weight. This suggests that the effect of the deficiency on this tissue might be independent of a change in body weight.

Zinc concentrations in serum, testes, and epididymides are shown in Table II. The serum Zn concentration in Zn-deficient rats was only about 30% of the values in *ad libitum* or pair-fed controls. Pair-feeding had no effect on serum Zn. Refeeding Zn-deficient rats the Zn-adequate diet for 2 weeks restored serum Zn to the normal range.

Although serum Zn in Zn-deficient rats was depressed by more than 65%, compared to the level in either control group, testicular Zn was depressed by only about 16%, but was significantly different ($P < 0.05$) from these groups. Pair-feeding did not affect the testicular Zn concentration. Refeeding Zn-adequate diets to Zn-deficient rats for 2 weeks restored testicular Zn to concentrations approaching those in the *ad libitum*-fed controls.

The epididymal Zn concentration was affected by both Zn deficiency and pair-feeding (Table II). Zinc deficiency reduced the epididymal Zn concentration to only 30% ($P < 0.002$) of the *ad libitum*-fed control value and to 45% of the pair-fed control value; both differences were highly significant ($P < 0.001$). The zinc concentration in the epididymides of pair-fed control rats was 65% of the *ad libitum*-fed control value and not significantly different ($P = 0.09$). Refeeding Zn to Zn-deficient rats for 2 weeks restored epididymal Zn to a level not different from that in the pair-fed controls, but different from that in the *ad libitum*-fed controls ($P < 0.006$). When pair-fed rats were returned to the *ad libitum* regimen for 2 weeks, the epididymal Zn concentration did not change from the initial values.

The number of sperm found in the epididymis was affected by treatment. Table III shows that the number of sperm in the epididymis of rats fed Zn-deficient diets for 4 weeks was only 6% of that in the *ad libitum*-fed controls and about 20% of that in the pair-fed group. The sperm count in the pair-fed group was only 30% of the control value. When the deficient rats were refed Zn-adequate diets for another 2 weeks, the sperm count did not change significantly. On the other hand, when pair-fed rats were refed *ad libitum* for this period, sperm count increased dramatically and was higher, but not

Table I. Effect of Zn Depletion and Repletion on Body Weight, Weight Gain, and Tissue Weights of Rats

	Depletion period: 4 weeks			Repletion period: 2 weeks	
	+ZnAL	−ZnAL	+ZnPF	−Zn/+ZnAL	+ZnPF/+ZnAL
Final body wt (g)	206 ± 6[a]	118 ± 4[b]	142 ± 8[c]	183 ± 5[a]	198 ± 8[a]
Weight gain (g/day)	4.4 ± 0.2[a]	1.4 ± 0.1[b]	2.3 ± 0.2[c]	5.0 ± 0.3[a,d]	4.2 ± 0.2[a]
Testis (mg)[e]	1192 ± 42[a]	569 ± 63[b]	807 ± 116[b,c]	839 ± 66[b,c]	1056 ± 104[a,c]
Testis (mg/100 g body wt)	585 ± 37	487 ± 64	558 ± 66	457 ± 34	531 ± 45
Epididymis (mg)	205 ± 12[a]	72 ± 7[b]	118 ± 21[b,c]	120 ± 10[b,c]	152 ± 17[c]
Epididymis (mg/100 g body wt)	101 ± 8[a]	62 ± 7[b]	81 ± 13[a,b]	65 ± 5[b]	77 ± 8[a,b]

Values are the mean ± SE of seven replicates/group. Means across groups with different letters ([a], [b], and [c]) are significantly different from each other at $P \leq 0.05$.

[d] Gain during repletion period only.

[e] Tissue values are based on wet weight of one organ each.

Table II. Effect of Zn Depletion and Repletion on the Concentration of Zn in Serum, Testes, and Epididymides of Rats

	Depletion period: 4 weeks			Repletion period: 2 weeks	
	+ZnAL	−ZnAL	+ZnPF	−Zn/+ZnAL	+ZnPF/+ZnAL
Serum Zn (μmol/liter)	24.6 ± 1.1[a]	8.3 ± 1.7[b]	24.3 ± 1.1[a]	24.7 ± 0.8[a]	23.1 ± 0.8[a]
Testes Zn (mmol/kg)[c]	2.61 ± 0.06[a]	2.12 ± 0.09[b]	2.54 ± 0.12[a]	2.36 ± 0.06[a,b]	2.61 ± 0.14[a]
Epididymal Zn (mmol/kg)	2.44 ± 0.31	0.74 ± 0.10	1.63 ± 0.21	1.28 ± 0.11	1.49 ± 0.17
Ln (epididymal Zn)	0.85 ± 0.14[a]	−0.34 ± 0.13[b]	0.46 ± 0.14[a,d]	0.22 ± 0.09[d]	0.34 ± 0.10[d]

Values are the mean ± SE of seven replicates/group. Means across groups with different letters ([a], [b], and [d]) are significantly different from each other at $P \leq 0.05$. For epididymal Zn, the analysis of variance was performed on ln-transformed data.
[c] Tissue values are based on dry weight.

Table III. Effect of Zn Depletion and Repletion on the Sperm Count in the Epididymis of Rats

	Depletion period: 4 weeks			Repletion period: 2 weeks	
	+ZnAL	−ZnAL	+ZnPF	−Zn/+ZnAL	+ZnPF/+ZnAL
Sperm count[a]	35.3 ± 4.3	2.2 ± 1.5	10.9 ± 1.1	3.5 ± 2.0	56.5 ± 16.2
Ln (sperm count + 1)[b]	3.54 ± 0.11[c]	0.65 ± 0.27[d]	1.14 ± 0.45[c]	0.88 ± 0.33[c]	3.37 ± 0.49[c]

Values are the mean ± SE of seven replicates/group. Means across groups with different letters ([c] and [d]) are significantly different from each other at $P \leq 0.05$.
[a] Sperm count equals the number of sperm found in 1 g of tissue multiplied by 1×10^{-6}.
[b] One was added to each value because some were zero.

significantly, than that in *ad libitum*-fed controls at 4 weeks.

Figure 1A shows the effects of Zn depletion and repletion on ACE activity in rat testes. ACE activity in the testes of Zn-deficient rats was only about 26% of that in the *ad libitum*-fed controls. Pair-feeding also depressed ACE activity slightly compared to that in the *ad libitum*-fed controls, but the difference was not significant. Refeeding Zn-adequate diets to Zn-deficient rats restored ACE activity to 65% of that in the *ad libitum*-fed rats. However, *ad libitum* feeding of pair-fed rats caused no change from the initial values.

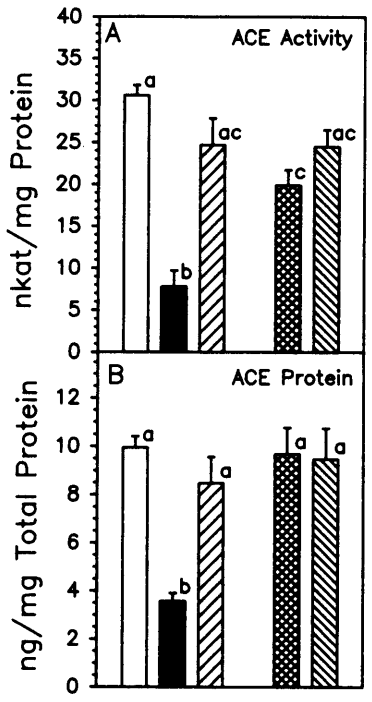

Figure 1. The effect of Zn depletion and repletion on Triton X-soluble ACE activity and ACE protein concentration in testes of rats. The bars represent the mean ± SE of seven replicates/group. □, +ZnAL; ■, −ZnAL; ▨, +ZnPF; ▩, −Zn/+ZnAL; ▧, +ZnPF/+ZnAL. Bars with different letters are significantly different from each other at $P \leq 0.05$.

Figure 1B shows the effect of Zn deficiency on the ACE protein concentration in the testis. The amount of reduction in the ACE protein concentration (64%) in Zn-deficient rats, compared to that in the *ad libitum*-fed controls, was similar to the change in enzyme activity (74%). Although refeeding Zn-adequate diets to Zn-deficient rats did not completely restore ACE activity to the level in the *ad libitum*-fed controls, the difference between the Zn-deficient and pair-fed controls was not significant. However, compared to either control group, the amount of immunoreactive ACE protein in testes of refed Zn-deficient rats was completely restored.

To determine if the effect of Zn deficiency on testicular enzymes was nonspecific, we determined the activity of a membrane-bound, non-Zn-dependent enzyme, γ-glutamyl transferase. The activity was determined in the same Triton X-solubilized fraction as ACE activity. Table IV shows that Zn deficiency *per se* had no effect on the activity of γ-glutamyl transferase. In addition, the data in Table IV show that the amount of protein in this fraction was not significantly affected by Zn deficiency.

A large part of ACE activity in the epididymides is

Table IV. Effect of Zn Depletion and Repletion on γ-Glutamyltransferase (γ-GT) Activity and Protein Concentration in the Triton X-Solubilized Particulate Fraction of Rat Testis

	Depletion period: 4 weeks			Repletion period: 2 weeks	
	+ZnAL	−ZnAL	+ZnPF	−Zn/+ZnAL	+ZnPF/+ZnAL
γ-GT activity (pkat/mg of protein)	94.9 ± 5.6[a]	106.1 ± 6.1[a]	97.9 ± 10.0[a]	68.4 ± 10.0[b]	63.0 ± 11.0[b]
Protein (mg/g of tissue)	15.9 ± 0.6[a]	15.6 ± 0.3[a]	16.3 ± 0.4[a]	19.9 ± 0.5[b]	19.3 ± 0.7[b]

Values are the mean ± SE of seven replicates/group. Means across groups with different letters ([a] and [b]) are significantly different from each other at $P \leq 0.01$.

derived from the testes, is found in the tubular fluid, and is soluble in buffer containing NaCl. We measured ACE activity in both salt-soluble and particulate fractions of this tissue. Figure 2A shows that ACE activity in the particulate fraction was not affected by Zn deficiency or pair-feeding. On the other hand, activity in the salt-soluble portion (Fig. 2B) was reduced to only 15% of that in the *ad libitum*-fed controls when rats were fed Zn-deficient diets. About 60% of the activity was lost when rats were pair-fed. Refeeding Zn to Zn-deficient rats increased soluble ACE activity only a small amount, and this was not significant. Returning pair-fed rats to the *ad libitum* regimen did not significantly change soluble ACE activity from the previous values.

Study 2. To determine the effects of age on the response of testicular ACE to Zn deficiency, the deficiency was initiated in rats at 22 and 30 days of age.

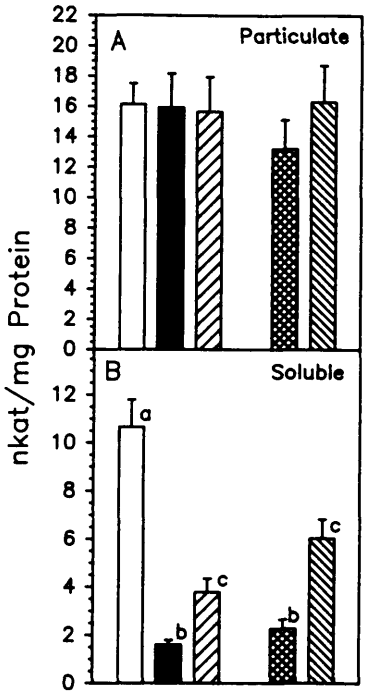

Figure 2. Effect of Zn depletion and repletion on ACE activity in Triton X-solublized particulate fraction and salt-soluble fraction of the epididymis. The bars represent the mean ± SE of seven replicates/group. □, +ZnAL; ■, −ZnAL; ▨, +ZnPF; ▩, −Zn/+ZnAL; ▧, +ZnPF/+ZnAL. Bars with different letters are significantly different from each other at $P \leq 0.05$.

Table V shows the results after 4 weeks. Results from Study 1 (row 2) are shown for comparison. Although signs of deficiency (slowed gain in body weight) began at nearly the same time (3–4 days on the deficient diet) in all groups, those rats that became deficient at 22 days of age were the most affected. Zinc deficiency as well as pair-feeding severely reduced both ACE activity and ACE protein concentration compared to those in the *ad libitum*-fed control group. Conversely, when rats were first fed the Zn-deficient diet at 30 days of age, there was no effect of the deficiency or pair-feeding on ACE activity. The ACE protein concentration was not measured in this age group. When 26-day-old rats (Study 1) were used, the results were intermediate between those deficient at 22 days of age and those at 30 days of age.

Discussion

Previous experiments in our laboratory showed that Zn deficiency in young rats reduced the activity of ACE in testes. With the use of an ELISA specific for ACE protein, we have shown in the present study that this reduction in activity is probably caused primarily by a reduction in the amount of ACE protein. The reduction in ACE activity (74%) in testes of Zn-deficient rats, compared to controls, was similar to the reduction in ACE protein concentration (64%). Zinc repletion of deficient rats restored the ACE protein concentration to control levels and ACE activity to 65% of the control value.

Table V. Effects of Age and Zn Depletion for 4 Weeks on ACE Activity and ACE Protein in Testes of Rats

Age when Zn deficiency initiated	+ZnAL	−ZnAL	+ZnPF
ACE activity (nkat/mg protein)			
22	29.0 ± 1.2[a]	3.8 ± 0.7[b]	2.2 ± 1.3[b]
26 (Study 1)	30.6 ± 1.2[a]	7.8 ± 0.6[b]	24.7 ± 2.7[a]
30	30.3 ± 1.1[a]	25.5 ± 3.2[a]	30.3 ± 1.2[a]
ACE protein (ng/mg of total protein)			
22	12.4 ± 0.8[a]	5.2 ± 0.4[b]	5.1 ± 0.5[b]
26 (Study 1)	10.0 ± 0.5[a]	3.6 ± 0.3[b]	8.5 ± 1.0[a]
30		Not done	

Values are the mean ± SE of seven replicates/group. Means across groups with different letters ([a] and [b]) are significantly different from each other at $P \leq 0.005$.

We also showed that these effects might be age dependent. When rats were presented with Zn-deficient diets near 3 weeks of age, the chances of developing low ACE activity and low ACE protein were considerably greater than those after this age. After 4 weeks of age, however, ACE activity in the Zn-deficient group was slightly lower than that in controls, suggesting that if the experiment had been run longer, the difference might have been greater. Much of the effect of the deficiency could have been caused by reduced food intake in the deficient rats. Restricting the intake of controls to that of the deficient group caused a marked reduction of both ACE activity and protein when rats were 22 days old, but not when they were older.

In the reproductive tract of the male rat, ACE is distributed heterogeneously into soluble and particulate-bound forms. ACE in testes is 98% particulate bound, and in the epididymis it is about 50% particulate bound and 50% soluble (11). In the progression of ACE expression during development, epididymal particulate ACE appears first, beginning at about 3 weeks of age. This is followed a week later with the appearance of testicular ACE. Two weeks after testicular ACE, the soluble enzyme in the epididymis appears (7).

There is evidence which suggests that most of the soluble ACE from the epididymis is derived from sperm. Wong and Uchendu (22) showed that ligation of the efferent duct of the testes completely eliminated sperm from the epididymal lumen and at the same time lowered luminal ACE activity by 75% compared to activity when ducts were not ligated. Strittmatter *et al.* (7) showed that hypophysectomy also depleted both testicular and soluble epididymal ACE. In addition, soluble ACE has immunological properties more similar to those of testicular ACE than to particulate epididymal ACE (11).

Our results lend some support to the suggestion that soluble ACE of the epididymis is derived from the testis by way of the sperm, but not entirely. ACE activity was depressed in the testes of Zn-deficient rats. However, in the epididymis, the particulate fraction of ACE activity was not affected by Zn deficiency, whereas activity in the soluble fraction was very low. This paralleled a very low sperm count in the epididymis. When Zn-deficient rats were refed Zn-adequate diets for 2 weeks, ACE activity in testes was restored to near normal, but activity in the soluble portion of the epididymis was not affected. Again, these changes coincided with a low sperm count in the epididymis. The lower activity in soluble epididymal ACE of the pair-fed group also corresponded to a smaller sperm count after 4 weeks. Although sperm count increased dramatically after 2 weeks of *ad libitum* feeding, soluble ACE activity only increased by about 40% over the previous value and was close to being significant ($P < 0.06$). The lag period was similar to that found by Strittmatter *et al.* (7) where the first appearance of ACE in the testes and the first appearance in the soluble portion of the epididymis were separated by 2 weeks.

Our studies also suggest that low ACE activity and low ACE protein concentration in testes of Zn-deficient animals might be caused by the failure of the spermatids to develop. ACE in testes was found primarily in the spermatids in developmental stages 1–8 (23, 24), and spermatid maturation has been shown to be affected by Zn deficiency. Barney *et al.* (25) showed histologically that Zn deficiency reduced the number of spermatids in the seminiferous tubules of rats. There were no observable sperm in the ducts of the epididymal head. Mason *et al.* (4) also showed that Zn deficiency caused damage to the seminiferous tubules and reduced sperm count in the epididymis. They found that if the damage was not too extensive, it could be reversed by restoring Zn to the diet. If, however, damage was severe, no reversal was achieved. Diamond *et al.* (5) also observed that testicular lesions caused by Zn deficiency could be reversed within 1 week of refeeding Zn-adequate diets. In the present study, the concentration of ACE protein and its activity were restored to near normal by refeeding Zn-deficient rats Zn-adequate diets for 2 weeks. However, this occurred only if the deficiency was initiated in rats at 26 days of age or older. If the deficiency were initiated in 22-day-old rats, enzyme activity and protein concentration were not restored after 7 days of repletion (data not shown).

In the multiple sequence of events in testicular development, where is Zn involved? Failure of exogenous gonadotropin to stimulate testosterone production in Zn-deficient rats prompted McClain *et al.* (26) to suggest that hypogonadism in Zn deficiency is the result of Leydig cell failure. However, we have shown that if Zn deficiency is initiated in prepubertal rats, the expression of ACE protein is severely reduced. The fact that most of the testicular ACE is located in the spermatids suggests that the initial insult of the deficiency might be in the developing germ cell. Whether fully active ACE is required in this process is still unknown (27).

Parts of these studies were presented at the Annual Meetings of North Dakota Academy of Sciences at Grand Forks, ND, in April 1992, and at the International Society for Trace Element Research in Humans Third International Conference and the Nordic Trace Element Society Fourth Nordic Conference in Stockholm, Sweden, in May 1992.

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