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Abnormal Zinc Metabolism in Unilateral Maldescended Testes of a Mutant Rat Strain (42379)

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Abstract. The status of zinc in a mutant rat strain with heritable maldescended testes was examined. In rats with unilateral maldescended testis, the ectopic testis consistently had decreased zinc content (121.0 ± 23.0 μg zinc/g dry wt), while the eutopic testis had zinc content similar to that of normal rats (182.0 ± 5.0 μg zinc/g dry wt). Uptake of zinc by the ectopic testis was comparable to normal. Sephadex gel chromatography showed greatly reduced zinc content of one of the endogenous zinc binding fractions with a mol wt of 30,000 of cytosol of the ectopic testis in spite of a near normal protein content. Incorporation of zinc-65 into this fraction was also shown to be greatly reduced in ectopic testis. Sodium dodecylsulphate–polyacrylamide gel electrophoresis demonstrated that a protein of 23,000 Da was greatly reduced in quantity. This 23-kDa protein in ectopic testis may play a role in reduced testicular function of the ectopic testis.
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The function of zinc in sexual development of the male has been studied for a long period of time. However, the exact role played by the element is still very poorly defined. Zinc is present in high concentration in male accessory sex organs (1, 2). Using radiotracer techniques it has been shown that rat testes and male accessory sex organs accumulate zinc from blood (1, 3). Zinc deficiency always results in hypofunction of the testes (4–7) and depressed testicular steroidogenesis (8, 9). Besides its effects on androgen metabolism, zinc directly interacts with steroid receptors and androgen binding proteins (10–16). Zinc metabolism of the sexual organs, on the other hand, is controlled by the action of the steroid hormones. Castration and administration of estrogen to male rats cause decrease of prostatic zinc level (17). Administration of testosterone, on the other hand, induces an increase in the concentration of prostatic zinc (18, 19). Previous studies with testicular feminization (tfm) rats showed that cryptorchid testes had drastically reduced zinc and alkaline phosphatase levels (20). Subsequent experiments with surgically induced cryptorchid testes in normal rats demonstrated a comparable decrease in testicular zinc content as observed in tfm rats (21). Whether this decreased zinc content of cryptorchid testes in tfm rats and in experimental cryptorchidism is caused by a similar factor, i.e., elevated environmental temperature of the testes, or by a genetic factor as occurred in tfm rats is further investigated in this study using another mutant rat strain with maldescended testis, the ect rats.

These rats, a mutant strain of the King–Holtzman hybrid rats, exhibit an autosomal recessive or sex-linked defect manifested as either unilateral or bilateral ectopic testes (22). The ectopic testis is found just cranial to the external inguinal ring in the subcutaneous connective tissue of the ventral body wall. There is no scrotum on the affected side. Affected testes, epididymides, and seminiferous tubules are reduced in size. These testes contain immature Sertoli and Leydig cells and exhibit arrested spermatogenesis (23, 24). The normal or eutopic testis of the ect rats with unilateral ectopic testis is anatomically and functionally normal (22–24).

Materials and Methods. *Animal acquisition.* The genetic mutant strain of King–Holtzman hybrid rats with unilateral maldescended testis, the ect rats from the Stanley–Gumbreck colony, and their normal littermates were obtained from the International Foundation for the Study of Rat Genetics and Pest Control (Oklahoma City, Okla.). Normal controls used in all experiments were normal littermates of the mutant strains to assure minimum genetic variability. The animals

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were kept in an air-conditioned and light-controlled room and fed *ad libitum* with water and Rodent Laboratory Chow (Ralston Purina Co.). The rats were numbered individually for random selection for experiments. Unless rats of specific age were required for the experiment, all rats used were 120 days of age with weights ranging from 386 to 445 g to avoid age-related variations.

Reagents and glassware. Reagent grade chemicals were purchased from Sigma Chemical Company (St. Louis, Mo.). Sephadex G-75 and electrophoresis chemicals were supplied by BioRad Laboratories (Richmond, Calif.). Zinc-65 in the form of $ZnCl_2$ (sp act 3.77 mCi/mg) was purchased from Amersham Corporation (Arlington Heights, Ill.). Triple deionized water was used throughout this study. Polyethylene labware was used whenever possible because of its generally low level of zinc contamination. All glassware and labware were cleaned by soaking in concentrated nitric acid for 24 hr and rinsed in double deionized water (25). Aqueous solutions were analyzed for zinc.

Quantitation of zinc and copper. For serum zinc measurement, blood was obtained, after the rats were anesthetized with ether, from the abdominal aorta and allowed to clot at 4°C for 2 hr in trace metal-free plastic tubes. Serum was separated by centrifugation at 4°C and stored at −20°C until analyzed. For tissue zinc measurement, the tissue was rinsed with double-deionized water, blotted dry with filter paper and weighed. The tissues were stored frozen at −20°C until analyzed. Serum samples were analyzed after appropriate dilution with 0.1 *N* nitric acid. Tissue samples were weighed to obtain the wet weight and then dried at 110°C for 3 to 5 days until a constant dry weight was obtained. Dry ashing was performed in a muffle furnace (Sybron) at 500°C for 24 hr, followed by wet ashing at 90°C after addition of 1 ml of concentrated nitric acid. The ashed material was reconstituted with 1 ml of 0.1 *N* nitric acid. Metal analysis was done by flame atomic absorption using a Perkin–Elmer 703 atomic absorption spectrophotometer equipped with deuterium background correction and an AS-1 autosampler. Copper in serum was analyzed by flameless atomic absorption using the same instrument equipped with an HGA 500 graphite furnace (26). Triple distilled water was used for all solution preparation. Standards were prepared from certified atomic absorption reference solutions (Fisher). Day-to-day variations were also checked using a standard made up of bovine liver obtained from the National Bureau of Standards. Samples were analyzed in triplicate. Quantity of radioactive zinc-65 was determined by counting in polypropylene counting vials with a Beckman 8000 gamma counter. Tissues were rinsed with physiological saline before counting.

Studies on testicular zinc uptake. Six ect rats with unilateral maldescended testis were used for this experiment. Zinc-65 (0.082 μmole) (20 μCi) was injected intraperitoneally. Twenty hours after the administration of zinc-65, the rats were sacrificed under ether anesthesia. The ectopic and the eutopic testes were removed, rinsed three times with cold physiological saline, and counted with a Beckman 8000 gamma counter. The radioactivity of both testes were expressed in counts per minute (cpm) per gram wet weight of the testis. The radioactivity was also expressed as percentage of the dose administered.

Identification of cytosolic zinc binding proteins. Testes were homogenized in ice-cold 0.01 *M* Tris–HCl, pH 8.6. The homogenate was centrifuged at 100,000*g* for 1 hr at 4°C. The supernatant (3 ml) was applied immediately to a Sephadex G-75 column (1.5 × 120 cm) equilibrated with 0.01 Tris–HCl, pH 8.6, with 10 m*M* β-mercaptoethanol and 0.01% sodium azide. Proteins were eluted with the equilibrating buffer at 4°C. Protein elution was monitored by measuring absorbance of each fraction at 280 nm and the zinc content of each fraction was determined by either atomic absorption spectrophotometry or radioisotope counting. The chromatograms for the elution of the zinc binding proteins were plotted. This procedure was repeated with the ectopic testes, the eutopic testes, and the normal testes of normal rats. The same elapsed time was maintained between sacrificing the rat and chromatographing of the testicular cytosol in all experiments to minimize variation due to instability of proteins.

The fractions corresponding to each zinc peak were pooled and assayed for protein by the method of Lowry *et al.* (27) and zinc by flame atomic absorption spectrophotometry.

The values of total amount of zinc and percentage of zinc applied recovered in each peak were calculated. The experiment was repeated two times for both types of testes with ectopic and eutopic testis chromatographed through the same column alternatively. The means and standard deviations of each peak in the abnormal and control runs were computed. The values of the abnormal and control runs were compared with the Student paired t test.

Sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of the 30-kDa zinc binding fraction. Fractions under the zinc peak corresponding to 30 kDa (peak 2) were pooled and concentrated by ultrafiltration through a YM10 membrane under nitrogen at 4°C with an Amicon cell (28, 29). The concentrated sample was incubated at 90°C for 5 min in sample buffer (0.62 M Tris–HCl, pH 6.8, 2% SDS, 4% sucrose, 0.001% bromophenol blue, and 5% β-mercaptoethanol). It was then diluted with 1 vol of 2× sample buffer, and electrophoresed through a Laemmli vertical slab polyacrylamide gel with 1% sodium dodecylsulphate (30). A 4% stacking gel at pH 6.8 and a 12% separating gel at pH 8.8 were used. Protein bands were stained with Coomassie blue R-250 (31). Protein standards were run in parallel with the samples. They were phosphorylase b, mol wt 94,000; albumin, mol wt 67,000; ovalbumin, mol wt 43,000; carbonic anhydrase, mol wt 30,000; trypsin inhibitor, mol wt 20,100; and α-lactalbumin, mol wt 14,000, all purchased from Pharmacia Fine Chemicals (Piscataway, N.J.).

Statistical analysis. The tissue metal concentrations and zinc-65 uptake results were analyzed for significant differences in the means of the control (normal or eutopic) or experimental (ectopic) groups by using the Student t statistic for a two sample test (unequal number of observations per group for normal controls) or for paired observations (sample size same when eutopic testes were used as control) using a preprogrammed Texas Instruments calculator (T1/59, Texas Instruments, Dallas, Tex.).

Results. *Testicular metal content.* Ectopic testes of the 120-day-old ect rats were similar in size to that of tfm rats (0.40 ± 0.065 g wet wt) and were much smaller than the normal testes. The normal eutopic testis of the ect rat, however, was comparable in size to the testes of normal rats (1.30 ± 0.08 g wet wt in eutopic testes compared with 1.40 ± 0.09 g wet wt in normal testes).

Table I shows the zinc and copper contents of serum and liver of ect rats and normal rats. No statistically significant difference was observed between ect rats and normals, suggesting that the ect rats were not deficient in zinc. Table II shows the zinc and copper content of

TABLE I. ZINC AND COPPER CONCENTRATIONS IN ECTOPIC RAT AND NORMAL RAT LIVER AND SERUM

	Liver		Significance
	Ectopic	Normal	($P < 0.001$)
Zinc (μg/g dry weight)	107 ± 8[a]	95 ± 5	N.D.[c]
Copper (μg/g dry weight)	17.4 ± 2.0	16.7 ± 0.9	N.D.
	Serum		
	Ectopic	Normal	
Zinc (μg/ml)	148 ± 18	142 ± 15	N.D.
Copper (μg/ml)	133 ± 27	134 ± 9	N.D.

Note. Animals were sacrificed under ether anesthesia. Blood was drawn from the abdominal aorta, allowed to clot at 4°C for 2 hr, and serum separated by spinning. Prior to analysis, serum was diluted with 0.1 N nitric acid. Liver was rinsed with physiological saline, blotted dry with Whatman 3MM paper, and weighed to obtain the wet weight. It was then dried to a constant weight and dry ashed in a Muffle furnace followed by wet ashing with 1 ml of concentrated nitric acid. The ashed material was reconstituted with 0.1 N nitric acid. Zinc and copper were analyzed by flame atomic absorption spectrophotometry. Results presented were obtained from analysis performed on seven animals of each group.

[a] Mean ± SD.

[b] N.D., no difference.

TABLE II. ZINC AND COPPER IN NORMAL RAT TESTIS AND ECTOPIC RAT NORMAL AND ECTOPIC TESTES

	Zinc (μg/g dry wt)	Significance ($P < 0.01$)	Copper (μg/g dry wt)	Significance ($P < 0.01$)
Normal rat—Testis	191 ± 10[a]	S.D.[b]	14.8 ± 1.5	N.D.[b]
Ectopic rat				
Normal—Testis	182 ± 5	S.D.[b]	13.3 ± 0.3	N.D.[b]
Ectopic—Testis	121 ± 23		14.2 ± 2.0	

Note. Experimental procedure was similar to that described under Table I. Twelve animals of each group were analyzed.
[a] Mean ± SD.
[b] Significant compared to the ectopic rat ectopic testis. S.D., significantly different; N.D., no difference.

the testes. Copper contents of the testes were very similar between normal rat testes and the testes of the ect rats. Zinc content of the eutopic testes (182 ± 5 μg zinc/g dry wt) was also similar to that of normal rat testes. The ectopic testes, however, had decreased zinc content (121.0 ± 23.0 μg zinc/g dry wt), comparable to that of the cryptorchid testes in tfm rats (20). Thus, there was zinc deficiency in the ectopic testis of ect rats in spite of a normal body zinc level and the only element affected was zinc.

Zinc uptake by ectopic testis of ect rats. Following intraperitoneal injection of 20 μCi of zinc-65 (20 hr), the radioactivity recovered in the ectopic and eutopic testis of the same rat was compared. Table III presents the results expressed as radioactivity retained by the testes per gram wet weight of the tissue as well as percentage of injected dose per gram wet weight. The amounts of radioactivity retained by the eutopic and ectopic testes were comparable in all the animals examined. The slight variation in percentage of radioactivity retained in different animals was probably due to the variation in age of the animals used as reflected by their weight (ranged from 386 to 445 g). Since the eutopic testes of ect rats had been shown to be structurally and functionally normal in previous studies (23–25) as well as in this study, this result suggested that uptake of zinc by the ectopic testis was normal and that the decreased zinc content of the testis was caused by some other factors.

Identification of zinc binding proteins in testicular cytosol. Cytosol of testes of normal rats and both the ectopic and eutopic testes of the ect rats were analyzed by column chromatography. Cytosol of normal testis gave typically three zinc peaks: peak 1 at the void volume (>70 kDa), peak 2 corresponded to 30 kDa, and peak 3 corresponded to 10 kDa (Fig. 1A). A variable fourth zinc peak sometimes appeared at the wash volume of the column. Figure 1B represents the chromatogram of the normal eutopic testis cytosol. It was

TABLE III. ZINC UPTAKE BY ECTOPIC AND EUTOPIC TESTIS OF ECT RATS

	Testicular wet weights (g)		Zn-65 (cpm/g wet weight)		% Dose/g wet weight	
Animals	Eutopic	Ectopic	Eutopic	Ectopic	Eutopic	Ectopic
1	1.53	0.60	16854	21289	0.20	0.25
2	1.53	0.49	15982	20578	0.19	0.24
3	2.14	1.15	20598	28478	0.24	0.34
4	1.57	0.78	19577	22782	0.24	0.28
5	1.57	0.77	18143	23578	0.22	0.29
6	1.59	0.85	17774	21287	0.22	0.26

Note. Ect rats were injected intraperitoneally with 20 μCi of zinc-65 (0.082 μmole). Twenty hours after zinc-65 injection, the testes were removed, weighed, and counted. Paired t test ($P < 0.001$), no difference between ectopic and eutopic testes.

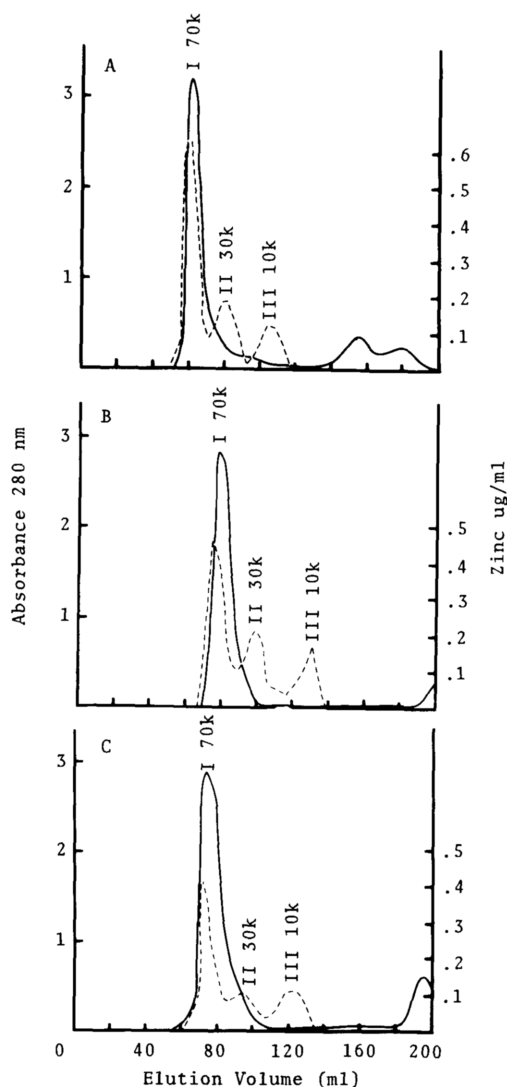

FIG. 1. Sephadex G-75 chromatogram of testicular cytosol. Testis was homogenized in 0.01 *M* Tris-HCl, pH 8.6. The supernatant (3 ml) obtained after centrifugation at 100,000*g* was applied to a Sephadex G-75 column (1.5 × 120 cm) equilibrated with 0.01 *M* Tris-HCl, pH 8.6, with 10 m*M* β-mercaptoethanol and 0.02% sodium azide. The proteins were eluted with the equilibrating buffer. Absorbance at 280 nm of each 4-ml fraction collected was measured. The zinc content of each fraction was determined by flame atomic absorption. (A) Cytosol of normal testis of normal rat; (B) cytosol of eutopic testis of ect rat; (C) cytosol of ectopic testis of ect rat. —, 280 nm absorbance; ---, zinc concentration.

similar to that of a normal testis. Figure 1C shows the chromatogram of the ectopic testis cytosol. Zinc peak 2 was greatly reduced. Analysis of the zinc content of each zinc peak in the testicular cytosol in the ectopic and eutopic testes showed that zinc peak 2 of ectopic testis contained less zinc than the control. The results are presented in Table IV. The zinc contents of zinc peak 1 and zinc peak 3 of the ectopic and eutopic testis were quite comparable. The zinc content of peak 2 of eutopic testis was always about twice of that of ectopic testis. The difference was significant at $P < 0.10$. Similar results were obtained when the values were expressed as percentage of zinc applied to the column recovered in each peak.

Uptake of Zn-65 by cytosolic zinc binding proteins. Zinc-65 (20 μCi) was administered to ect rats intraperitoneally. The rats were sacrificed at 6 and 20 hr after zinc administration. Cytosol of the ectopic and eutopic testes of the rats at both time points was analyzed by column chromatography. Zinc-65 radioactivity of each eluted fraction was measured. The chromatograms are shown in Figs. 2A and 2B. Comparable amounts of zinc-65 radioactivity in 3 ml cytosol were applied to the column for both ectopic and eutopic testis at each time point. At both time points, percentages of total applied radioactivity retained by peak 1 in both ectopic and eutopic testes were comparable. Peak 2 of eutopic testis cytosol retained more zinc-65 radioactivity than that of ectopic testis at both 6 hr (35.2% of applied dose for eutopic testis compared with 13.0% of applied dose for ectopic testis) and 20 hr (21.5% of applied dose for eutopic testis compared with 5.6% of applied dose for ectopic testis). There was a decrease in the percentage of zinc-65 retained by peak 2 of both types of testis with time and the decrease was bigger for ectopic testis than for eutopic testis. Peak 3 of ectopic testis always retained more zinc-65 than that of eutopic testis. These results indicated that even though the overall zinc uptake of the ectopic testes was comparable to that of the eutopic testes, the amount of zinc retained by zinc peak 2 was reduced in ectopic testes. The reduction in testicular zinc content of ectopic testes was apparently due to abnormality of the zinc peak 2 constituents.

Identification of missing protein by SDS–PAGE. Fractions under zinc peak 2 of both the ectopic and eutopic testes were pooled and concentrated by Amicon ultrafiltration. After incubation with sample buffer, the samples

TABLE IV. ZINC CONTENTS OF THE ZINC PEAKS OF TESTICULAR CYTOSOL OF ECT RATS CHROMATOGRAPHED THROUGH A SEPHADEX G-75 COLUMN

Zn peak	Type of testis	Total Zn in peak (μg)	Difference ($P < 0.1$)	Percentage of applied Zn recovered in peak	Difference ($P < 0.1$)
1	Eutopic	4.25 ± 0.47[a]	N.D.[b]	46.4 ± 1.9	N.D.
	Ectopic	4.21 ± 0.68	N.D.	50.8 ± 1.8	N.D.
2	Eutopic	2.02 ± 0.54	S.D.	22.0 ± 4.4	S.D.
	Ectopic	1.07 ± 0.33	S.D.	12.8 ± 2.3	S.D.
3	Eutopic	1.39 ± 0.35	N.D.	15.1 ± 2.8	N.D.
	Ectopic	1.94 ± 0.40	N.D.	24.2 ± 8.2	N.D.

Note. The fractions under each zinc peak were pooled and the zinc content was determined by flame atomic absorption spectrophotometry. The percentage of zinc recovered in each peak was calculated. The experiment was repeated twice for both types of testes with the ectopic and eutopic testicular cytosol chromatographed through the same column alternatively.

[a] Mean ± SD.

[b] Statistical difference as calculated by the Student paired *t* test. N.D., no different; S.D., significantly different.

were analyzed by SDS–PAGE. The Coomassie blue-stained protein pattern was shown in Fig. 3. A protein band corresponding to 23 kDa was greatly reduced in the zinc peak 2 of ectopic testis.

Discussion. Results of this study showed that ectopic testis similar to cryptorchid testis has decreased zinc content. Ectopic rats and tfm rats exhibit different genetic abnormalities. In tfm rats there is no evidence of a reproductive tract other than bilateral small cryptorchid testes with well-developed Leydig cells but arrested spermatogenesis and end organ insensitivity to endogenous or exogenous androgens manifested by an absence of androgen dependent differentiation (32). In ect rats the epididymides and seminiferous tubules of the ectopic testis are present albeit reduced in size (23, 24). Androgen binding activity is also detected in the ectopic testis and epididymis of the ect rat even though it is reduced compared with that of normal rats (23, 24). The major similarity between the ectopic testis in ect rats and cryptorchid testis in tfm rats is their elevated environmental temperature. The environmental temperature of cryptorchid and ectopic testes is 37.5 and 35.5°C, respectively, while the normal scrotal temperature is 32.5°C (23, 24). In experimental cryptorchidism, localization of normal testis in the abdomen for 2 weeks causes no change in concentration of androgen binding protein (ABP) per mg protein, although there is a marked and progressive decrease in ABP content per testis in a recent experiment (33). Zinc content of the testis is also reduced in experimental cryptorchidism (21). These facts suggest that the testis specific zinc deficiency in ect rats and tfm rats could be due to elevated environmental temperature of the testes. The cause of this testicular zinc deficiency by temperature is not well defined in this study. Reduced quantity of the 23-kDa protein in zinc binding peak 2 might be related.

Ectopic testes of the ect rat showed substantial reduction in testicular zinc content when compared with the eutopic testes. Taking into consideration the percentage of the total testicular zinc found in peak 2 (Fig. 1, Table IV), the lower zinc content of this peak accounts for about 50% of the difference between ectopic and eutopic testes. About 15% of the difference can be accounted for by a slight reduction in zinc content of peak 1. These results appear to indicate that the decreased zinc content observed in ectopic testes might be related to abnormal protein eluted in peak 2. This is also supported by the observation that uptake of zinc-65 by peak 2 was greatly reduced in ectopic testes. Analysis of the peak 2 proteins by SDS–PAGE revealed the drastic reduction of 23-kDa protein in ectopic testes. This 23-kDa protein is therefore a likely candidate responsible for the aberrant zinc status of ectopic testes.

Testicular zinc binding proteins have been the subjects of several reports. Besides carbonic anhydrase and alkaline phosphatase which are

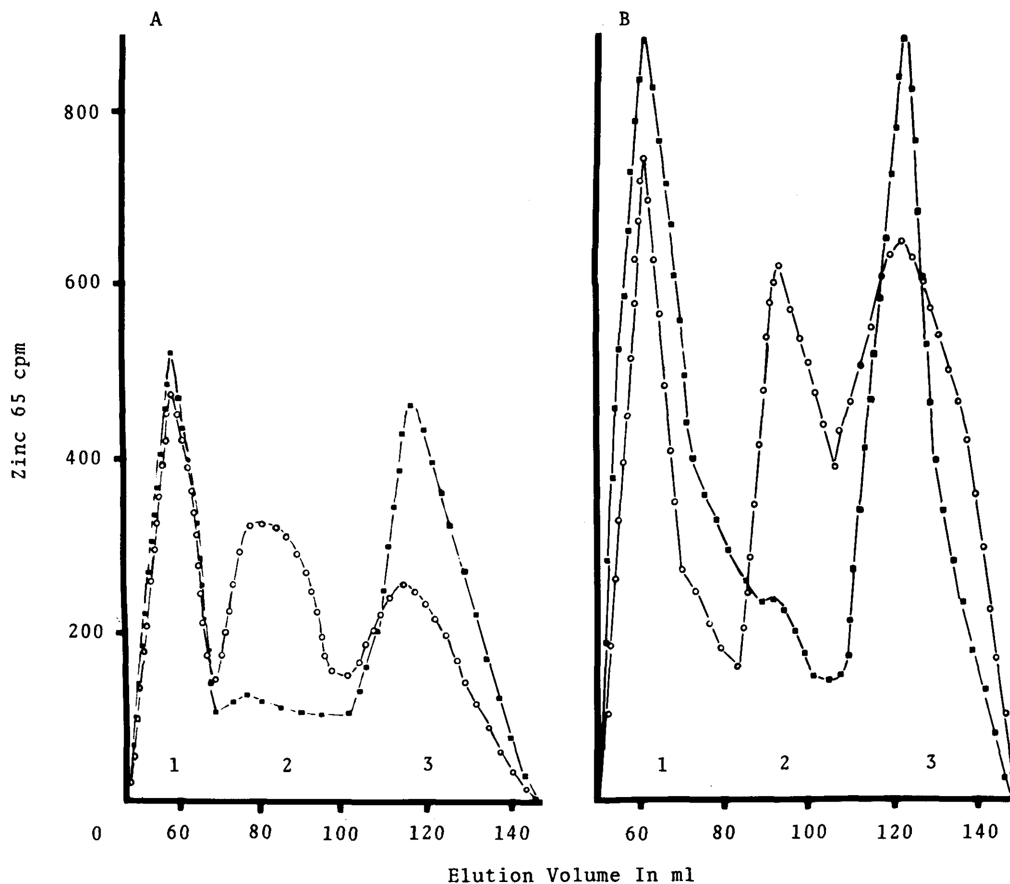

FIG. 2. Uptake of zinc-65 by cytosolic zinc binding proteins. Rats were sacrificed 6 and 20 hr after intraperitoneal administration of zinc-65. Testicular cytosol (3 ml) of the eutopic and ectopic testis containing comparable amounts of zinc-65 was chromatographed through a Sephadex G-75 column (1.5 × 120 cm) as described in the legend of Fig. 1. Radioactivity of each fraction was counted. (A) 6 hr after zinc-65 administration; (B) 20 hr after zinc-65 administration. (○), cytosol of eutopic testis; (■), cytosol of ectopic testis.

known testicular cytosolic zinc binding proteins Brady and Webb reported the presence of metallothionein in rat testes (34). This was later disputed by Waalkes *et al.* who detected three major zinc binding proteins with the major heat-stable one having a mol wt of 25,000 and none of them being metallothionein (35, 36). Earlier, Chen and Ganther had reported a testicular cadmium-zinc binding protein of 30 kDa which is unstable even at 4°C under nitrogen. This protein is tissue specific and is also absent in rooster testis (37). More recently the same group reported that the major component of this protein is a subunit of mol wt of 22,000 (38). Whether this subunit is similar to the reduced 23-kDa protein described in this report is not clear. Chen and associates detected the cadmium-zinc binding protein by *in vitro* labeling of testicular cytosol with radioactive zinc and cadmium. In another report the same investigators reported the *in vivo* labeling of a 30-kDa testicular cadmium-zinc binding protein after loading the rat with 0.6 mmole of Zn-65/kg for 24 hr followed by 0.012 mmole of Cd-109/kg for 30 min (39). Since both zinc and cadmium are known to be able to induce protein synthesis, especially metallothionein (40), whether this *in vivo* labeled cadmium-zinc binding protein is identical to the *in vitro* labeled 30-kDa protein has to be confirmed. The 23-kDa protein described in this report, on

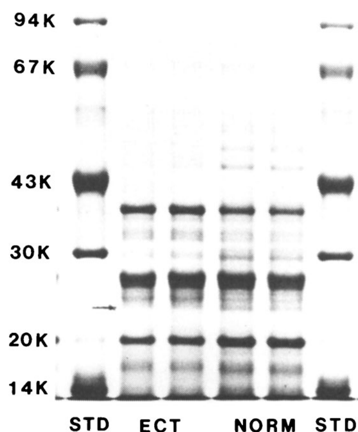

FIG. 3. Sodium dodecylsulphate–polyacrylamide gel electrophoresis of 30-kDa zinc peak. The fractions in zinc peak 2 with mol wt of 30,000 were pooled and concentrated by Amicon ultrafiltration. The concentrated sample was incubated with sample buffer for 5 min at 90°C. Protein (15 μg) was loaded to each lane. Electrophoresis was done with a 12% polyacrylamide gel slab with 0.1% sodium dodecylsulphate. Proteins were stained with Coomassie blue. S: standards were phosphorylase b, mol wt 94,000; albumin, mol wt 67,000; ovalbumin, mol wt 43,000; carbonic anhydrase, mol wt 30,000; trypsin inhibitor, mol wt 20,100; and α-lactalbumin, mol wt 14,000. NORM, normal eutopic testis; ECT, ectopic testis. The arrow on the left indicates the position of the 23-kDa band.

the other hand, is detected by following the elution of endogeneous zinc; i.e., it has endogenously bound zinc. Kinoshita and Ganther had suggested that the 30-kDa cadmium-zinc binding protein might participate in testicular zinc metabolism (38). Chausmer *et al.* have shown that calcitonin can regulate tissue zinc homeostasis in rats (41). A recent study provides evidence for the existence of the calcitonin receptor on testicular Leydig cells (42). A temperature-related change of the 23-kDa protein reported here, or the 30-kDa protein reported by Kinoshita and Ganther, or calcitonin–receptor interaction might result in abnormal testicular zinc metabolism. Which of these is the actual mechanism involved in abnormal zinc metabolism in ectopic and cryptorchid testes needs to be further investigated.

The interaction between zinc and androgen metabolism also deserves further investigation. Tfm rat testes are completely devoid of androgen action. Participation of androgen in testicular zinc metabolism is blocked. Ect rats have testes with structurally normal albeit immature Sertoli and Leydig cells and seminiferous tubules, but function abnormally when they are ectopic (23). Especially in the ect rats with unilateral maldescended testis, both testes are under normal androgen action but only the eutopic testis (normal side of the ect rat as contrast to the ectopic side) functions normally. The alteration of androgen binding protein activity in ectopic testis has been suggested to be caused by the increased temperature of the ectopic testis (23, 26). However, it has been suggested that Leydig cells and Sertoli cells do not degenerate in cryptorchid testes (43, 44). More recently, it has been shown that Sertoli cells are biologically more active at body temperature than at scrotal temperature (45). The observation that the production of ABP is decreased in Sertoli cells from cryptorchid as opposed to scrotal testes (46) thus cannot be assumed to be caused by the effect of the difference in temperature on Sertoli cells alone. The possibility of zinc being a causative factor in such a phenomenon cannot be neglected. In fact, Meftah *et al.* had reported that the total number of ABP sites per pair of testes for both dihydrotestosterone and testosterone were decreased in zinc-deficient rats in comparison to pair-fed and *ad libitum* fed controls (47). However, due to the reduction in testicular size of zinc deficient rats, expression of the results in terms of picomoles of ABP per milligram cytosolic protein or per gram of testis showed an increase instead of a decrease in ABP in zinc-deficient rats. This reversal of the change in zinc-deficient rats is probably an artifact due to the manipulation of the data and does not reflect the actual situation. A recent study also demonstrated a decrease in the concentration of cytosolic androgen receptor sites in the ventral prostate of zinc-deficient rats (48).

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