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The Distribution of Zinc in the Subcellular Fractions of the Rhesus Monkey Testis

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ABSTRACT

The zinc content in the testis of sexually immature, adult, and efferent duct-ligated adult rhesus monkeys was determined by atomic absorption spectrophotometry. Zinc content (μg/g wet wt) was found to be high in adult testis (165.9) when compared to immature (68.9) or efferent duct-ligated (104.2) animals. Analysis of subcellular fractions revealed that the concentration of zinc (expressed in relation to protein) was maximum in the microsomal fraction. The possible significance of this trace metal as a constituent of membrane proteins and enzymes, and as an activator of mitochondrial function in testis, is discussed.

Index Entries: Zinc, in monkey testis; zinc, in subcellular fractions of testis; zinc, in immature monkey testis; testis zinc, normal versus efferent duct ligation; monkey testis.

INTRODUCTION

The close relationship of testicular zinc content to endocrine and reproductive state of the testis was recognized by early investigators (*1*). An adequate supply of dietary zinc is essential for normal spermatogenesis in the rat (*2*) and in man (*3*). Some cases of idiopathic male infertility with low plasma levels of testosterone can be successfully treated by oral

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administration of zinc (4). Since the nonhuman primate is a natural animal model in the study of human reproduction, a study was carried out to determine testicular zinc content and its subcellular distribution pattern in adult rhesus monkey with a view to understanding the possible physiological role of zinc in the male gonad.

In lower mammals, efferent duct ligation is known to induce changes in testicular physiology and morphology (5–7). Congenital occlusion of vasa efferentia is reported to result in testicular atrophy in man (8). A study was, therefore, carried out to determine testicular zinc content following efferent duct ligation in monkeys and to compare it with that in sexually immature animals.

MATERIALS AND METHODS

Animals

Immature (2.5–3.0 kg, 12–15 mo old) and adult (8–10 kg) male rhesus monkeys of the Institute's primate colony were used in the present study. They were maintained in air-conditioned quarters (24 ± 1°C) under uniform husbandry conditions. In four adult animals, efferent ducts were ligated close to the testis. Care was taken not to damage the vascular supply of testis and epididymis. The animals were subjected to postoperative antibioic therapy for 5 d. After resting for 90 d, the animals were killed and the testes were dissected out. Similarly testes from six intact adult and four immature animals were dissected out and small portions of organ were fixed in Bouin's fluid for histological examination. Serial paraffin sections were stained with hematoxylin and eosin.

Preparation of Testicular Fractions

Weighed samples of testis tissue were homogenized (50 mg/mL) in 0.25*M* sucrose solution, using an Ultra-Turrax TP 18/2 N disintegrator in cold, and their centrifugal fractionation was carried out as described by Conchie and Hay (9) to obtain nuclear, mitochondrial, microsomal, and cytosolic fractions. Zinc was determined in the total homogenate and in the subcellular fractions by atomic absorption spectrometry (Perkin Elmer Model No. 5000), following the method described by Snaith et al. (10). Protein was measured by the procedure of Lowry et al. (11), using bovine serum albumin as standard.

Results were analyzed by Student's *t* -test for the determination of statistical significance.

RESULTS

It will be evident from the results presented in Table 1 that the zinc content per unit weight was significantly higher in adult monkey testis as

TABLE 1
Zinc Content in the Testis of Rhesus Monkey

Animal status/zinc content	Adult	Efferent duct ligated	Immature
μg/g wet wt	165.9 ± 9.3 (12)[a]	104.2 ± 5.9 (8)	68.9 ± 7.95 (8)
μg/mg protein	1.35 ± 0.07 (12)	0.916 ± 0.06 (8)	0.696 ± 0.11 (8)

[a]Mean ± SE with the number of tissue samples used in parentheses.
Zinc content on a tissue or protein basis: adult vs efferent duct-ligated/immature. $P < 0.001$.

compared to that of immature animals. Efferent duct ligation caused a marked reduction in zinc level of the testis. Results presented in Table 2 reveal that zinc concentration was highest in the nuclear sediment followed by cytosolic and mitochondrial fractions. In animal tissues it is found to be complexed to organic ligands, especially proteins (*12*). In view of this, zinc concentration expressed on the basis of protein level in the different subcellular fractions, offers a better comparison though this comparison by itself does not reflect its function. Calculated on the basis of protein level, zinc concentration was highest in microsomal fraction, followed by nuclear and cytosolic fractions.

Histological examination of adult testis revealed all the stages of spermatogenesis. In efferent duct-ligated animals, only spermatogonia and Sertoli cells were present in the seminiferous tubules. In one animal primary spermatocytes were also present in several tubules. The Leydig cells did not reveal any morphological changes from those of the intact testis. In immature testis spermatogenesis did not progress beyond spermatogonial stage.

DISCUSSION

The low zinc content in the testis of sexually immature monkeys is in agreement with previous findings in human testis, where its concentration increases at puberty and reaches a maximum at the age of 36–40 yr

TABLE 2
Zinc Content in the Subcellular Fractions of Adult Monkey Testis

Subcellular fraction/zinc content	Nuclear	Mitochondrial	Microsomal	Cytosolic
μg/g tissue	54.29 ± 3.27 (8)[a]	29.89 ± 1.97 (8)	21.93 ± 2.20 (8)	33.95 ± 1.52 (8)
μg/mg protein	1.66 ± 0.10 (8)	0.99 ± 0.07 (8)	4.36 ± 0.43 (8)	1.33 ± 0.06 (8)

[a]Mean ± SE with the number of estimations in parentheses.
Zinc content on a tissue basis: nuclear vs mitochondrial/microsomal/cytosolic, $P < 0.001$; zinc content on a protein basis: microsomal vs nuclear/mitochondrial/cytosolic, $P < 0.001$.

when the functional activity of the organ is highest (*13*). Following intraperitoneal injection into the rat, a major portion of the zinc is found to be incorporated into spermatids (*14*). It has also been observed that zinc content per unit weight of rat testis increases with the appearance of spermatids in the prepubertal period (*15*), suggesting an important role for zinc in the development of spermatids. The reduction in zinc content of testis following efferent duct ligation, therefore, appears to be caused by depletion of spermatids.

Recent studies have indicated that zinc has a special function in lipoproteins and proteins associated with biomembranes (*12*) and this may be the reason for high zinc content in the microsomal fraction of testis, an organ that exhibits a high mitotic activity. It is also pertinent to mention that monkey spermatozoa are rich in zinc (*16*) and its level is particularly high in the lipoproteins of sperm cells (*12*). The involvement of zinc as a prosthetic metal in certain metalloenzymes is also well recognized (*17*). Though little is known about microsomally bound zinc metalloenzymes, at least one important microsomal enzyme in testis, 17β-hydroxysteroid dehydrogenase (*18*) is known to be affected under zinc-deficient conditions, (*19*), as revealed by a decreased conversion of androstenedione to testosterone in men.

Its high concentration in the nuclear fraction may be attributed to its role in the structural integrity of DNA (*20,21*). A protein with high affinity for zinc has been reported to be present in rat testis cytosol (*22*) though its localization in the seminiferous tubules is not known. The uptake of (^{35}S)-cysteine by both the nucleus and cytoplasm of ram spermatids (*23*) and the association of zinc with cysteine-rich proteins in the rat and bull spermatozoa (*24,25*) is indicative of a critical role played by zinc-containing proteins in the differentiation of the spermatid and the organization of sperm structures.

In rat liver cells, zinc stimulates the activities of mitochondrial enzymes (succinic dehydrogenase, glutamate dehydrogenase, cytochrome oxidase, and ATPase) and increases ATP concentration in the cytosol, suggesting that zinc taken up by mitochondria stimulates oxidative phosphorylation in hepatic mitochondria (*26*). It is interesting to note that the extensive internal reorganization that the mitochondria undergo during spermiogenesis "resemble the changes seen in isolated mitochondria when the metabolism is altered from slow respiration to active oxidative phosphorylation" (*27*). Further studies are required to verify the precise role played by zinc in testicular functions in general and spermatid differentiation in particular.

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