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Immunohistochemical Demonstration of Metallothionein in the Rat Epididymis and Spermatic Cord

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ABSTRACT—We studied the immunohistochemical localization of metallothionein (MT) in the epididymis and spermatic cord of male Wistar rats. In the head of the epididymis, no MT immunoreaction of epithelial cells in the ductuli efferentes or ductus epididymidis was observed, but basal cells of the ductus epididymidis showed a positive immunoreaction for MT. In the body of the epididymis, a few epithelial cells in the ductus epididymidis had a positive immunoreaction for MT, but no positive immunoreaction was observed in the basal cells. In the tail portion near the body, basal cells in the ductus epididymidis showed a positive immunoreaction for MT while the epithelial cells had a negative one. In the portion near the spermatic cord, both the epithelial and basal cells in the ductus epididymidis had a positive immunoreaction for MT. In the spermatic cord, basal cells and some epithelial cells had a positive immunoreaction for MT. The localization of MT was observed mainly in the cytoplasm of the cells, with some nuclei of the cells having a positive immunoreaction. Different immunoreactions for MT were demonstrated in the epithelial and basal cells of the epididymis and spermatic cord, which suggested that epithelial cells function differently in various portions of the epididymis.

INTRODUCTION

Metallothionein (MT) is a known metal-binding protein with a low molecular weight, approximately 6,000 MW, and an ability to bind closs II-B metals, such as zinc and cadmium [1]. It has been identified in a variety of organs, including the liver, kidneys, prostate, and testes [1–4]. MT is induced in response to various physiological stimuli and endogenous factors [5]. Although the exact functions of MT are not clearly known, it has been considered to play a role in the detoxication, storage, and metabolism of heavy metals [6].

The epithelial cells of the human and rat prostate secrete MT [3, 7, 8] and zinc, an action which correlates with infertility [9]. Recently, MT was reported to be localized in the spermatogenic cells [10, 11], but few studies on MT in the epididymis have thus far been undertaken. The purpose of the present study was to demonstrate the localization of MT in the epididymis and spermatic cord as part of an investigation of the function of MT as it relates to sperm.

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MATERIALS AND METHODS

Male Wistar rats (10 weeks old) were purchased

from Imai Experimental Animals (Saitama, Japan) and were anesthetized by ether. The tissues examined by immunohistochemical staining were obtained from the epididymis (head, body, and tail) and spermatic cord.

Preparation of tissues for immunohistochemical examination

All tissues were fixed in 10% buffered formalin solution for 48 hours and embedded in paraffin. Specimens were cut at a thickness of 3 μm and mounted on glass slides. Staining was performed using an avidin-biotin peroxidase complex method. Sections were deparaffinized and incubated in 0.1% trypsin solution for 30 minutes at 37°C. They were then immersed in 0.5% (5 mM) periodic acid solution for 10 minutes in order to inhibit endogeneous peroxidase, and incubated with normal goat serum for 20 minutes to block the non-specific binding sites. Primary antibody was mixed with a 200-fold concentration of anti-MT-1 and ascaris antigen to inhibit non-specific reaction to ascaris, because anti-MT-1 antibody was obtained from rabbit antiserum boosted by MT-1 emulsion coupled to ascaris antigen with glutaraldehyde [7]. The slides were layered with primary antibody for 2 hours at room temperature and washed with 0.01 M PBS buffer (pH 7.2). The secondary antibody was applied for 1 hour and sections were again washed with PBS. They were treated with ABC complex for 30 minutes and washed with PBS. Sections were submerged in 0.05% diaminobenzidine tetrahydrochloride in 0.05 M Tris buffer (pH 7.6) to which H_2O_2 (0.01%) has been added just before use. These reagents were prepared using a VECTASTAIN ABC KIT (VECTOR Laboratories, Burlingame, USA). Subsequent counterstaining was performed with Mayer's hematoxylin. The specificity of the staining reaction was determined in a prior absorption of anti-MT antibody with pure liver MT antigen (MT-1 and/or MT-2) (Sigma Chem. Co., USA) and omission of primary antibody from the procedure.

RESULTS

Immunohistochemical control

As shown in Figure 1, the use of primary antibody that had been absorbed with pure rabbit liver MT-1 and MT-2 did not result in any specific immunoreaction in the epididymal head or tail or in the spermatic cord. Use of non-immune serum

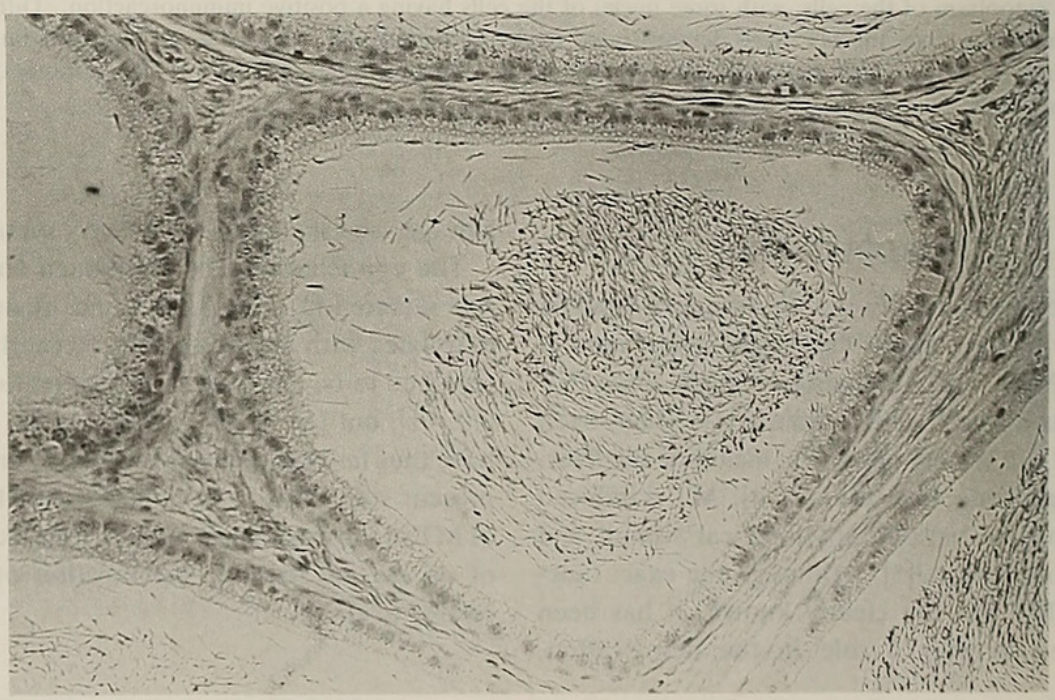

FIG. 1. Control section of the ductus epididymidis in the tail portion treated with preabsorbed primary antibody. Immunoreaction for MT was negative in the epithelial and basal cells (×220).

and PBS did not result in any immunoreaction in any sections.

Immunohistochemical findings

The head of the epididymis. This portion contained two ducts, the ductuli efferentes and ductus epididymidis. No immunoreaction for MT was observed in the epithelial cells of the ductuli efferentes (Fig. 2). In the ductus epididymidis, basal cells showed positive immunoreaction for

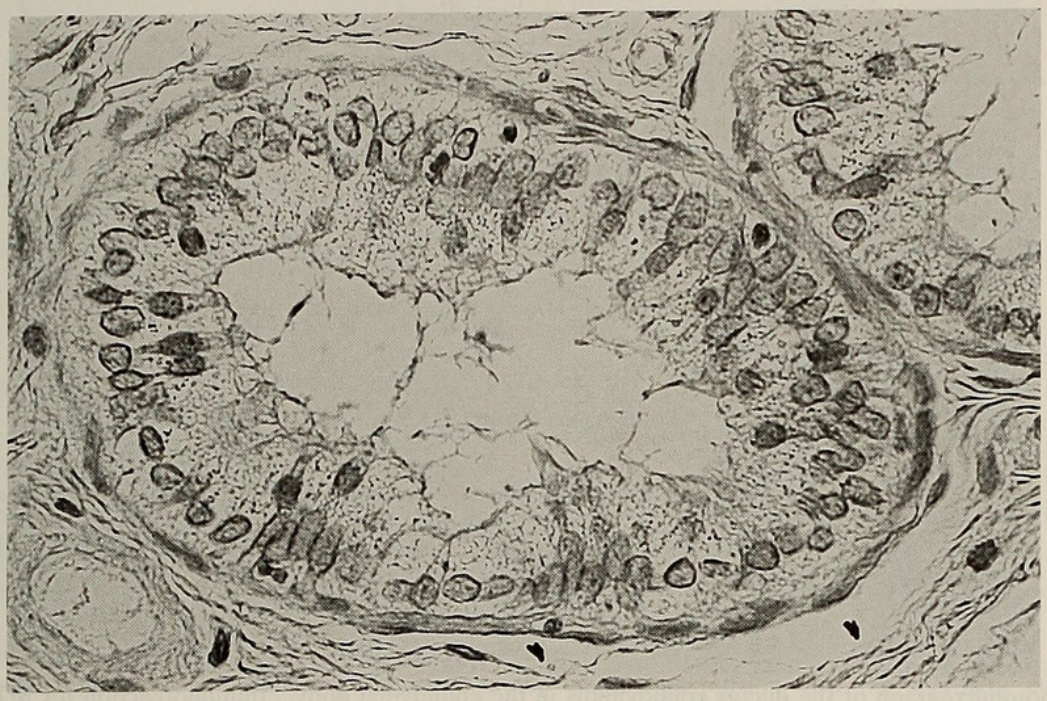

FIG. 2. Photomicrograph of the ductuli efferentes. Immunoreaction for MT was negative in the epithelial and basal cells and in connective tissues (×560).

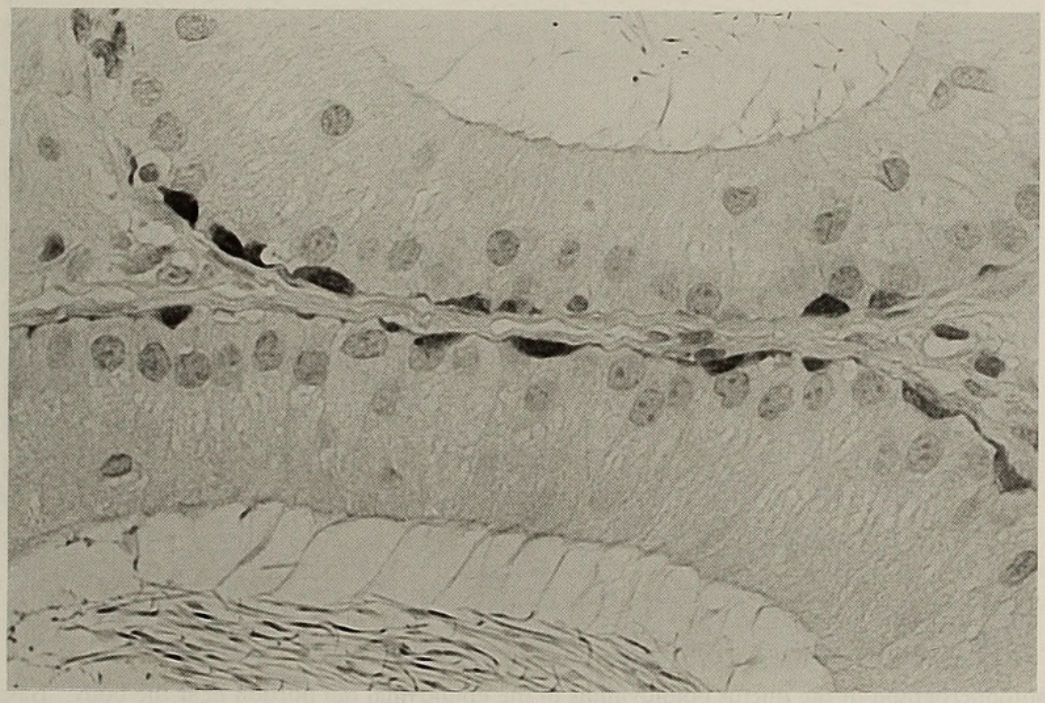

FIG. 3. Photomicrograph of the ductus epididymidis in the epididymal head. Immunoreaction for MT was found in the basal cells, but not in the epithelial cells or connective tissues (×560).

MT, but epithelial cells and connective tissues showed a negative one. MT immunoreaction was localized mainly in the cytoplasm and partly in the nuclei of epithelial cells (Fig. 3).

The body of the epididymis. A few epithelial cells of the ductus epididymidis, about one or two cells per one section of the ductus epididymidis, showed a positive immunoreaction for MT, but

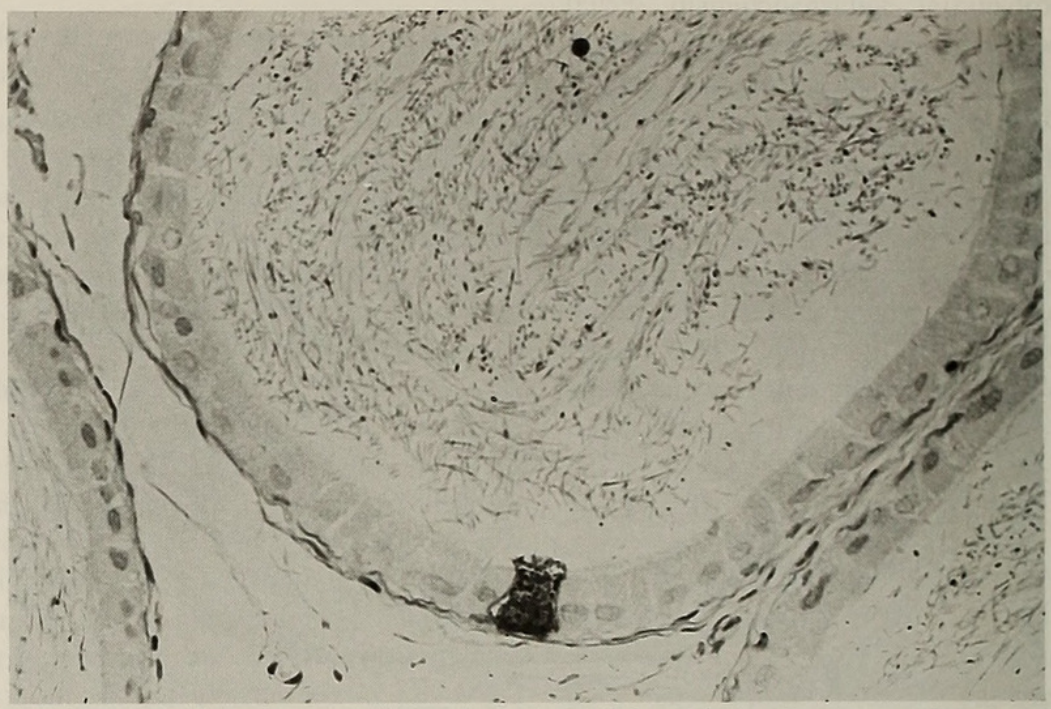

FIG. 4. Photomicrograph of the ductus epididymidis in the epididymal body. Immunoreaction for MT was found positive in one epithelial cells, and was localized mainly in the cytoplasm. The basal cells, sperm and fluid in the lumen, and connective tissues were negative (×350).

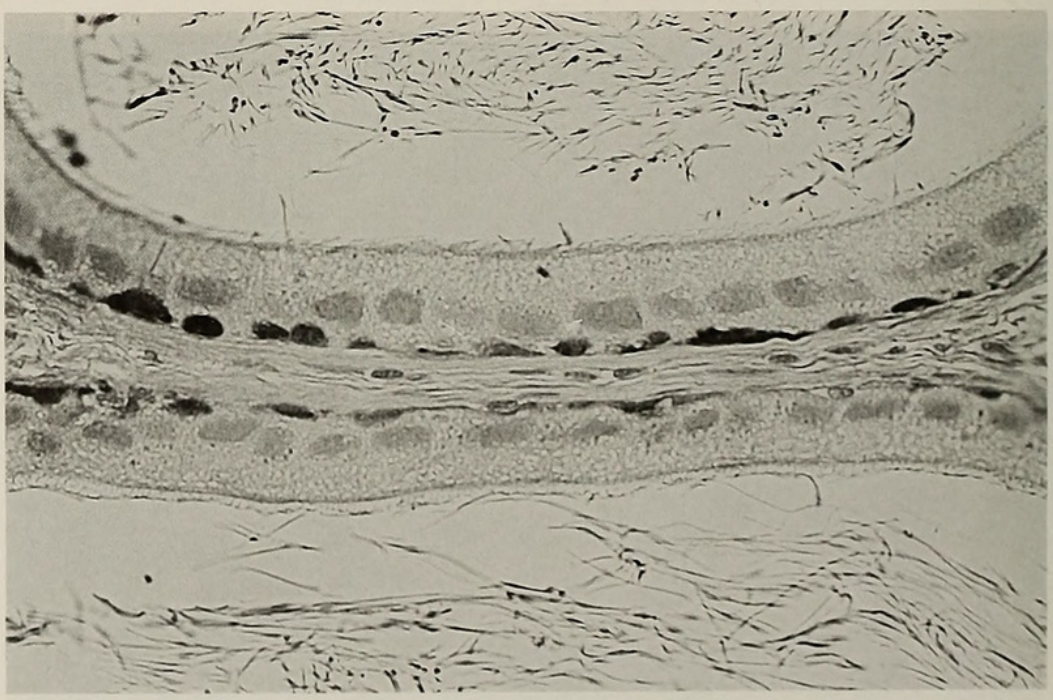

FIG. 5. Photomicrograph of the ductus epididymidis in the epididymal tail portion near the body. Basal cells had a positive immunoreaction for MT, but epithelial cells and connective tissues and sperm in the lumen were negative (×560).

most epithelial cells were nagative. The basal cells, sperm and fluids in the lumen, and connective tissues had no immunoreaction. MT immunoreaction was localized mainly in the cytoplasm, which appeared as fine granular immunostaining (Fig. 4).

The tail of the epididymis. In the portion near the body, basal cells of the ductus epididymidis

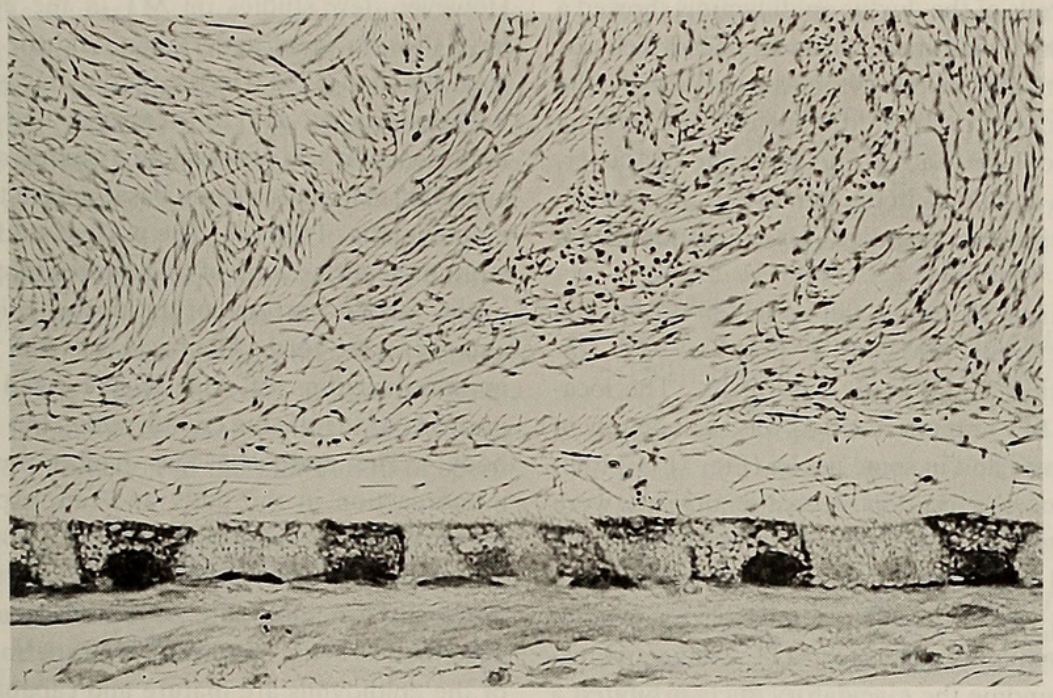

FIG. 6. Photomicrograph of the ductus epididymidis in the epididymal portion near the spermatic cord. Immunoreaction for MT was found strongly positive in the epithelial and basal cells, and was localized mainly in the cytoplasm, especially the apical area, and partly in the nucleus. Connective tissues and sperm in the lumen were negative (×560).

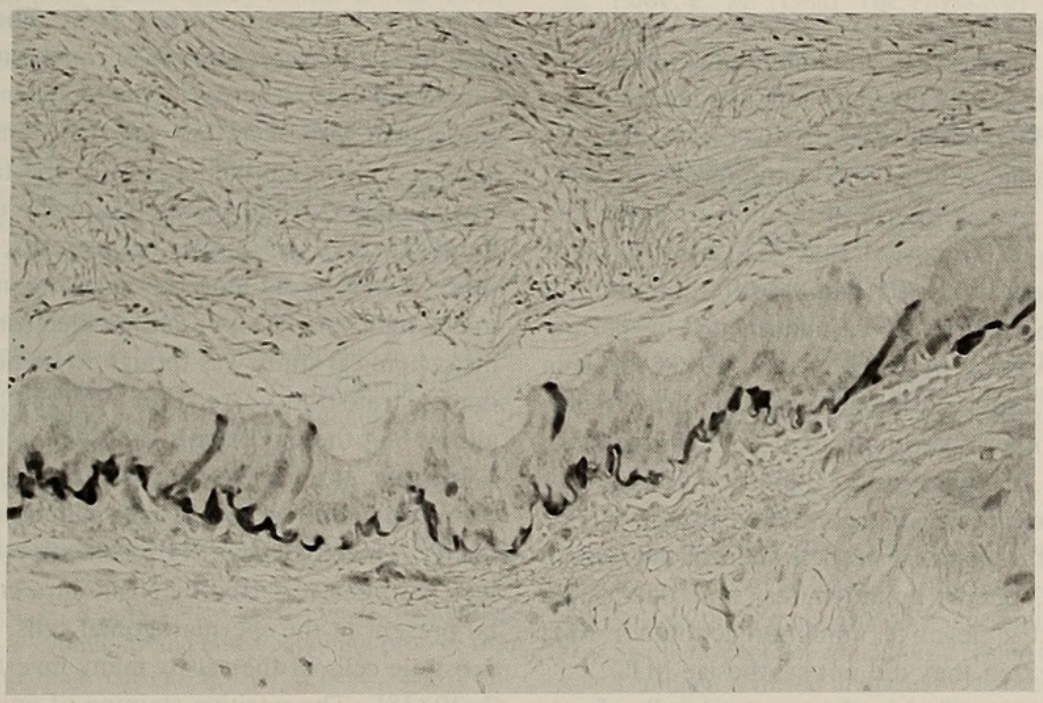

FIG. 7. Photomicrograph of the ductus deferens. Immunoreaction for MT was found positive in the basal cells and some tall epithelial cells. Connective tissues and sperm in the lumen were negative (×350).

exhibited a positive immunoreaction for MT, but epithelial cells showed a negative one. The sperm and fluids in the lumen and the connective tissues exhibited a negative immunoreaction (Fig. 5). In the portion near the spermatic cord, the epithelial and basal cells of the ductus epididymidis showed a strong positive immunoreaction for MT, which was localized mainly in the cytoplasm, especially the apical area, and partly in the nuclei. The staining in the cytoplasm had a fine granular character. The sperm and fluids in the lumen and the connective tissues were negative for immunoreaction (Fig. 6).

Spermatic cord. Some tall epithelial cells of the ductus deferens showed a positive MT immunoreaction as did many basal cells. The localization of MT immunoreaction was seen mainly in the cytoplasm and in some nuclei (Fig. 7). Epithelial cells of the portion near the urethra were mostly negative. The connective tissues and the sperm and fluids in the lumen were negative. The basal cells in the ejaculatory duct also showed a positive immunoreaction for MT. Connective tissues surrounding the ejaculatory ducts exhibited a negative immunoreaction.

DISCUSSION

The presence of MT in rat testes was reported by Nolan and Shaikh [12], and immunohistochemically demonstrated by Danielson [4] and Nishimura *et al.* [10]. MT immunoreaction was observed in the spermatogenic cells in the seminiferous tubules, but not in mature sperm in the lumen. Recently, De *et al.* [11] reported that MT mRNA which accumulated after the initial differentiation of primary spermatocytes was maintained in spermatids, and was shown to be present at low levels in interstitial, spermatogonial, and matured sperm cells by Nothern blooting and in situ hybridization. Therefore, they suggested there being a role for MT in the process of spermatogenesis. In this study, matured sperm in the lumen of the ductuli efferentes, ductus epididymidis, and ductus deferens had a negative immunoreaction for MT, suggesting that they did not synthesize MT.

MT was identified in the prostatic cells of rats and humans, and was shown to secrete into the prostatic fluids [3, 7, 8]. The seminal fluids mainly consisted of those derived from the testis and epididymis, the prostate, and the seminal vesicle. MT is known to bind to zinc, an action which is reported to correlate with infertility [9, 13]. However, few studies on MT in the epididymis have thus far been undertaken, and the physiological function of MT in the epididymis is unclear.

Nishimura *et al.* [10] reported that MT immunoreaction was observed in a limited number of epithelial cells and in some basal cells in the ductus epididymidis of the rat. The present study demonstrated the differences of immunoreactions for MT in the various portions of the ductus epididymidis and spermatic cord. The basal cells had a positive immunoreaction for MT in the head and tail portions of the ductus epididymidis, but not in the body. The epithelial cells had a positive immunoreaction in the body and tail, especially in many of the epithelial cells in the tail portion near the spermatic cord. The spermatozoa first to leave the testis are incapable of fertilization and are described as being immature. The sperm in the tail of the epididymis are mature and capable of fertilization [14]. The charactristic feature of maturation is a change in the surface of the sperm, as well as the presence of glycoprotein secreted from the epididymal epithelial cells which has been reported to bind to the sperm surface [15]. The principal cells has numerous cisternae of rough endoplasmic reticulum and well-developed Golgi apparatus associated with secretory function, and the MT localized in the epithelial cells of the tail portion was suggested to be secreted into the epididymal fluids [16, 17]. In this study, however, the secretion of MT could not be clearly demonstrated under light microscopy, and an electron immunohistochemical study was though to be necessary to demonstrate the correlation between the sperm and MT in the epididymis.

In this study, the localization of MT was observed mainly in the cytoplasm and partly in the nuclei. Histologically, MT localization in the nucleus has been reported by Nishimura *et al.* [10] to be present in the spermatogonial cells, and in the prostatic cells of the rat by many investigators [3, 7, 10, 18]. The positive staining of epithelial and basal cell nuclei might conceivably result from

contamination of nuclear proteins by the MT transposed during tissue preparation. However, no nuclei had ever shown a positive immunoreaction in the epididymis, reducing the likelihood of this explanation. Recently, large molecules containing the nuclear location signal sequence have been reported capable of being transported into the nucleus crossing the nuclear envelope of mammalian cells [19, 20]. This suggests that MT may also be transported into the nucleus and act as an enzyme activator by donation of zinc bound to it.

In summary, the localization of MT and differences of immunoreaction for MT in the various portions of the ductus epididymidis were demonstrated. Many epithelial cells in the tail had a positive immunoreaction for MT, and MT was suggested to function in the maturation of sperm.

ACKNOWLEDGMENTS

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