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Morphofunctional Aspects of Rat Leydig Cell Development

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In the present study the rat testes on postnatal days 5, 10, 15, 20, 24 , 27 and of mature Wistar rats, received single i.p. injection of ethane dimethane sulphonate (EDS) were used. The ultrastructural characteristics of the LC, 3β hydroxysteroid dehydrogenase (3βHSD), and glucose-6-phosphate dehydrogenase enzyme activities were studied. The dynamics of appearance and intensity of enzymes investigated corresponded with postnatal structural differentiation of the LC. The restoration of new LC population after EDS administration repeats the process of normal LC development with a similar time range. The results obtained strengthen the view for the differentiation processes of the LC during the postnatal development.

Key words: Leydig cells, ultrastructure, enzymohistochemistry, EDS

Introduction

The postnatal development of the Leydig cells (LC) in mature, differentiated cell forms with low proliferation ability involves the processes of proliferation, differentiation and testosterone production. It has been reported that during rat development two LC populations existed: fetal type LC, producing androgens required for the fetal masculinization and postnatal LC [9]. The fetal LC originate prenatally from mesenchymal-like precursor cells situated in the testicular interstitium, one part of which transform into rounder, testosterone-producing fetal LC, while others remain spindle shaped and differentiate after birth into the mature LC [9]. The fetal type LC rapidly regreses during the first three weeks after birth [13]. It should be noted that the adult LC derive not from the fetal type, but from the undifferentiated mesenchymal-like precursor cells or stem cells. The embryonic origin of Leydig stem cells is still disputable. There are data in this direction providing evidence that the mesonephric mesenchymal cells in the interstitium are a potential source of LC stem cells [3]. During the ontogenesis the interstitial mesenchymal cells form mixed population of stem cells from which derived also peritubular myofibroblasts, testicular macrophages and interstitial fibroblasts [2]. The difficulty to identify the Leydig stem cells is caused by lack of specific markers.

Based on data reported by H a r d y et al. [7], by 14 postnatal day Leydig stem cells proliferate and transform into progenitor cells, which are an intermediate stage in the LC development and could be found in rat testis between days 14 and 28 after birth. The LC progenitors differentiate morphologically into immature LC between days 28 and 56 postpartum which divide once and then transform into adult type LC.

Ethane-1, 2-dimethanesulphonate (EDS) is a unique toxin with cytotoxic action confined almost exclusively to adult, but not immature, rat LC [16, 12]. EDS selectively decreases or eliminates both basal and LH-stimulated testosterone production *in vivo* and *in vitro*, thus providing a model in which there is a complete loss of LC within the testis [12, 14]. It has been shown that a single dose of EDS injected into adult rats caused a temporary impairment of fertility, reduced levels or loss of serum and intratesticular testosterone and elevated pituitary secretion of LH and FSH as a result of the destruction of existing LC [11, 20, 16]. During the first two days after EDS application, the LC population in the rat may be eliminated by macrophages [5] and subsequently two to three weeks new LC appeared and regenerated completely eight to ten weeks after treatment, apparently from proliferating interstitial mesenchymal-like precursor cells [11, 18].

The purpose of our study was to establish the morphological and enzymehistochemical features of rat LC during the postnatal development and of newly formed LC population after treatment with EDS.

Material and Methods

Testes of Wistar rats (n=16) on days 5, 10, 15, 20, 24 and 27 after birth and of adult rats (n=7), received single i.p. injection of EDS (75mg/kg body weight) were used as material. The experimental animals were killed 14, 21 and 30 days after initial EDS administration.

Electron microscopy — testicular fragments were fixed in phosphate-buffered 2.5% glutaraldehyde and postfixed in 1% osmium tetraoxide. Tissue was dehydrated in ethanol and embedded in Durcupan (Fluka). The ultrathin sections were contrasted with lead citrate and uranyl acetate.

Electronograms were made on a Philips-CM 12.

Enzymohistochemistry — on fresh cryostat sections (5 μm thick) the enzyme activities of 3β hydroxysteroid dehydrogenase with substrate dehydroepiandrosterone [15] and glucose-6-phosphate dehydrogenase with substrate glucose-6-phosphat [8] were studied. *Controls.* Sections were incubated in medium without the specific substrate for the corresponding enzyme.

Results

The electron microscopic observation showed that in rat testicular interstitium on the 5th and 10th postnatal day abundant fetal type Leydig cells with large size and high density of the cytoplasm could be seen. The fetal LCs were recognizable by their spherical nucleus with dense chromatin, lipid droplets, well-developed smooth endoplasmic reticulum (SER) and mitochondria with mainly tubular cristae. Strong 3βHSD and glucose-6-phosphate dehydrogenase activities were found in the LC at these stages of postnatal development. Between 15 and 24 postnatal days the number of fetal type LC progressively decreased and structural features of their

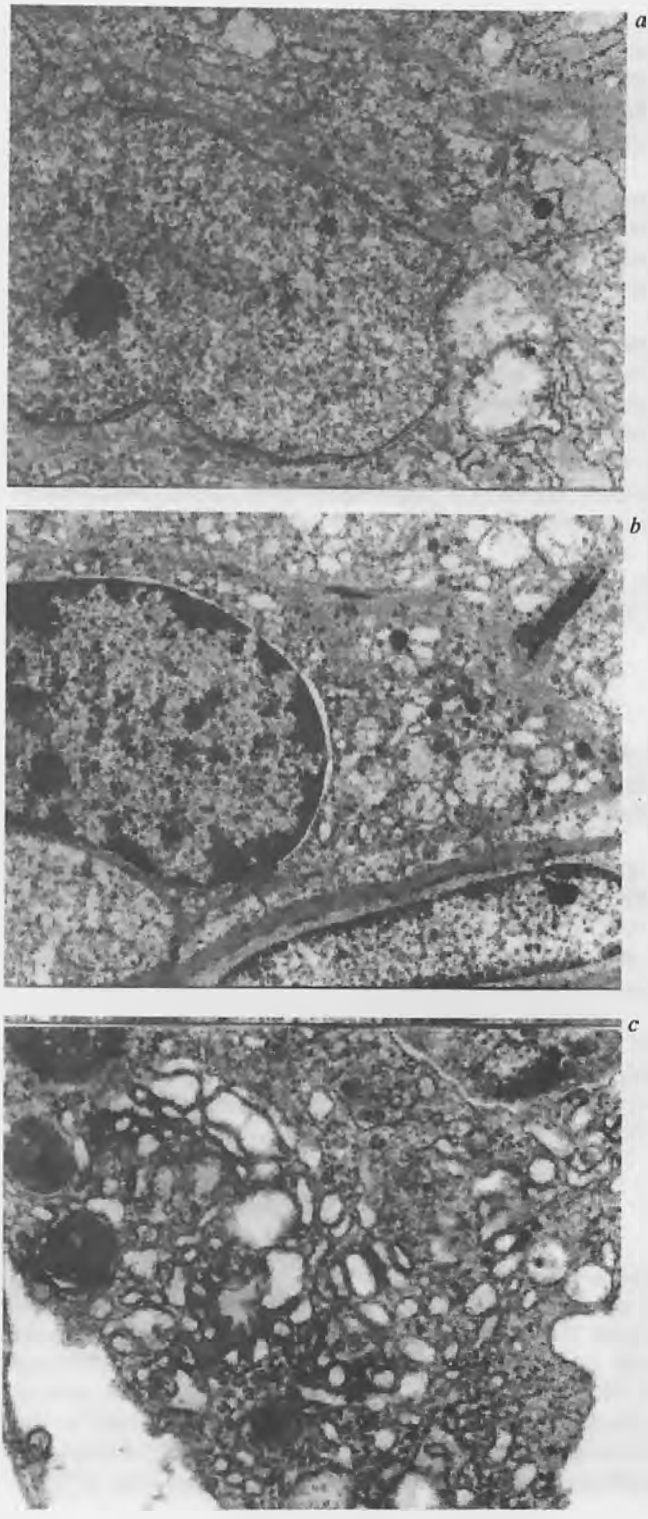

Fig.1. Electron micrographs
a — ultasructure of progenitor type LC (postnatal day 15) without lipids and well- developed SER (× 9600)
b — ultrastructure of immature LC (postnatal day 27). Numerous lipid droplets, steroidogenic type mitochondria and well-developed SER are evident in the cytoplasm (× 12 400)
c — ultrastructure of adult type LC (30 day after EDS). An abundance of smooth endoplasmic reticulum, Goldgi complex and tubular mitochondria was observed (× 19 000)

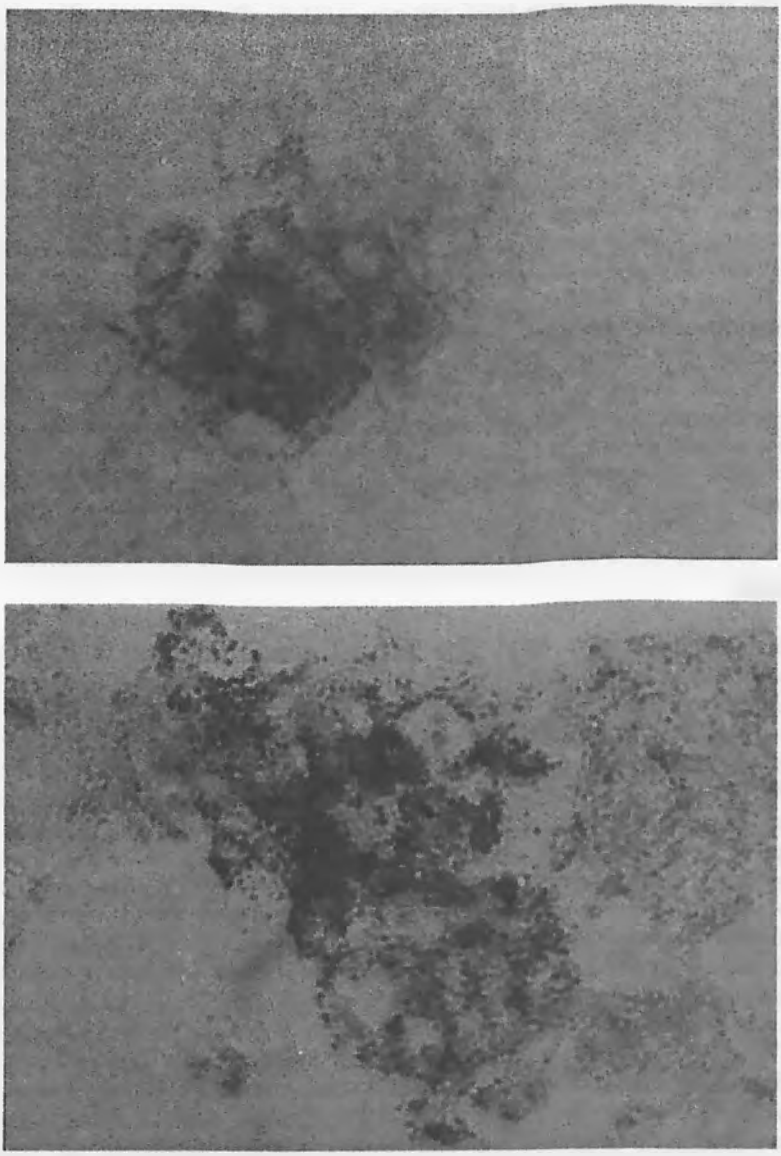

Fig. 2. Histochemical visualization of 3βHSD enzyme activity
a — in the immature Leydig cells (postnatal day 20) (× 400)
b — in the newly formed LCs 21 days after EDS treatment (× 400)

involution could be observed — volume reduction, nuclear condensation and vesicular form of smooth endoplasmic reticulum. In the intertubular space a few elongated or spindle shaped LC occurred whose ultrasructure corresponded to that of progenitor type LC — small cytoplasmic volume, no lipids and absence of well-developed SER (Fig. 1 — *a*). In comparison with the fetal type LC, 3βHSD and glucose-6-phosphate dehydrogenase enzyme activities in the LC progenitors were

reduced. On the 20th day after birth the appearance of interstitial cells with similar electron microscopic pattern to those of immature type LC were observed. In contrast to LC progenitors, immature LC were larger and rounder, with lipid droplets. Their cytoplasm was tightly packed with tubular cisterns of SER, pleomophic steroidogenic type mitochondria and rough endoplasmic reticulum (RER), (Fig. 1 — *b*). They were intensively stained for 3βHSD (Fig. 2 — *a*) and glucose-6-phosphate dehydrogenase activities (Fig. 3 — *a*). Two weeks after EDS administration the electron microscopical observation revealed spindle shaped progenitor type LC with small cytoplasm and well developed RER. The progenitors showed 3βHSD and glucose-6-phosphate dehydrogenase activities with low staining intensity. Between 21 and 30 days after treatment with EDS electron microscopic analysis demonstrated that immature type LC predominate in the interstistial space. A strong activity for 3βHSD (Fig. 2 — *b*) and glucose-6-phosphate dehydrogenase (Fig. 3 — *b*) was found in the cytoplasm of immature LCs. On day 30 after EDS adult type LCs can be observed between the seminiferous tubules. They were recognized by their abundant SER, steroidogenic type mitochondria, decline in lipid droplets (Fig. 1 — *c*). and intensively staining for 3βHSD and glucose-6-phosphate dehydrogenase activities.

Discussion

It has been reported that fetal type LC secreting androgens during the fetal period, progressively reduce in number and involute postnatally [9]. Our findings confirmed this fact, as well as other publication, in which the fetal LC were described as cells with numerous lipid droplets, well-developed SER and strong 3βHSD enzyme activity [4, 6, 13]. We found involution markers in the cytoplasm of some of the fetal LC such as vesiculation of SER and membrane whorls. Our results showed that between 15 and 24 postnatal days, besides the reduced number of fetal LC, elongated or spindle shaped progenitors were observed, which corresponds to previously data for the presence of LC progenitors in rat testis between days 14 and 28 postpartum [7]. In the present study moderate to low 3βHSD and glucose-6-phosphatdehydrgenase enzyme activities were detected in the LC during this period. Because of using of NAD as a cofactor, the activity of 3βHSD is dependent of glucose-6-phosphate dehydrogenase. In this regard, the developmental changes in glucose-6-phosphate dehydrogenase enzyme activity are similar to 3βHSD intensity pattern. The mechanism by which LC progenitors produce androgen when they lack well developed SER and have weak 3βHSD enzyme activity is still obscure. An explanation in this direction is that the LC progenitors maybe have mitochondrial form of steroidogenic enzymes [1]. The present results confirmed the established transformation of progenitors into immature type LC with abundant SER and lipid droplets and increase in steroidogenic enzyme activities in them between postnatal days 28 and 56 [4, 6, 17]. Following EDS administration, we found the first 3βHSD enzyme activity on the day 14 after treatment and strong increasing labeling intensity for this enzyme in the newly formed LC on days 21 and 30. Our data confirm the results by T e e r d s et al. [19] indicating 3βHSD enzyme activity as a marker for LC differentiation after EDS treatment and provide additional evidence for the similarity between steroidogenic enzyme pattern in rat LC during the postnatal development and after EDS. Therefore, the increase in steroidogenic enzyme activity occurs in tandem with the structural features of steroidogenic differentiation.

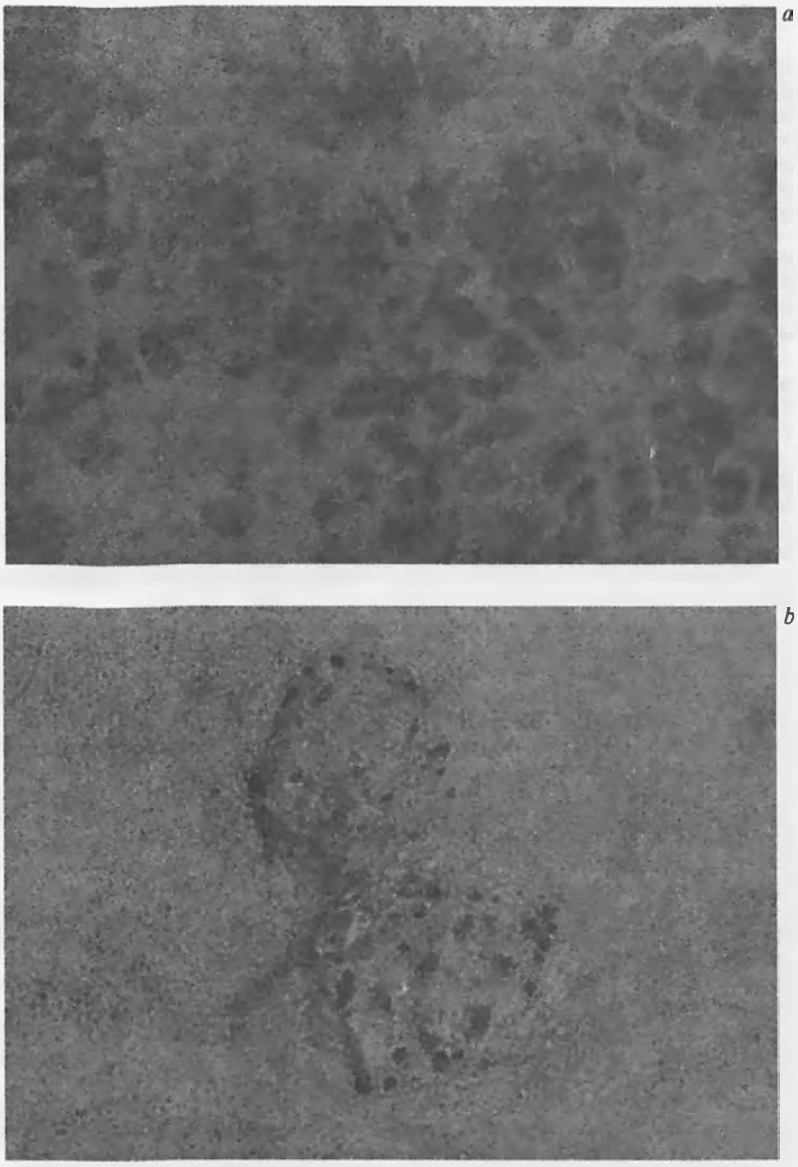

Fig. 3. Histochemical visualization of glucose-6-phosphate dehydrgenase enzyme activity
a — in the clusters of interstitial LCs on day 24 postpartum (× 400)
b — in the newly formed LCs 21 days after EDS treatment (× 400)

In conclusion, as far as the regeneration of LC repopulation in adult testis after EDS treatment repeats the prepubertal LC development, the results obtained strengthen the view for the differentiation processes of LC in rat testis.

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