The Fine Structure of Testicular Interstitial Cells in the Mouse Administered with Vitamin E

By

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Introduction

Since the reproductive disturbances of rats associated with vitamin E-deficiency were first reported by Evans & Bishop ('22), subsequently histological changes of testes induced by vitamin E-deficient diet in rats were described by many authors. In those reports, some authors noted that testicular degeneration induced by vitamin E-deficiency was found only in seminiferous tubules and not recognized in interstitial cells (Van Wageningen, '25; Mason, '26; Evans & Burr, '27; Kudrjaschov, '30, '31; Juhasz-Schaffer, '31, '32; Nelson, '33; Griesbach, et al. '57), while on the other hand several authors observed histological changes in interstitial cells of rats in such condition (Marchesi, '35; Ringsted, '36).

Furthermore, recently Fujino & Yoshioka ('62) described that vitamin E administration activates the function of interstitial cells in mature mice. As described above, studies concerning the relationship between vitamin E and histological changes of interstitial cells of testes are not definite yet. In this study mature male mice were administered with vitamin E and the interstitial cells of their testes were investigated with electron microscope with respect to their ultrastructural changes. On the basis of this study, the author presents in this paper the close relationship between vitamin E and the function of interstitial cells of testes in mature male mice.

Materials and Methods

A total of 20 mature male mice of dd strain were used in this study. They were divided into two groups. The first group was 10

normal intact mice. The second group was 10 mice injected daily with 10 mg of vitamin E (dl- α -Tocopheryl acetate prepared by EISAI Co., Ltd.) for 20 days. Each animal was killed by decapitation. Bits of testes were dissected out quickly and fixed in buffered isotonic OsO_4 solution at 4°C for 2 hours. After fixation, the tissues were washed in Ringer's solution, rapidly dehydrated in ethanol series and then embedded in Epon 812 according to Luft's method. The thin sections were cut on a JUM-4 ultramicrotome (Japan Electron Optics Laboratory Co., Ltd.), and then stained in a 0.5% uranyl acetate alcohol solution for 1 hour. Sections were viewed in HUA 11 electron microscope (Hitachi Ltd. Tokyo Japan).

Observations

Normal Testicular Interstitial Cells (Fig. 1, 2, 3)

These cells are generally oval or irregularly rectangular in shape. Between adjacent cells many interstices (Fig. 1) are clearly observed. The cell surface exposed to the interstices is sometimes covered with microvilli and in some cases small hollowed cell surface studded with numerous microvilli is recognized at perivascular space (Fig. 3).

The mitochondria are moderate in size, commonly oval or rod-shaped and their internal matrices exhibit abundant irregular tubular cristae (Fig. 2).

In cytoplasm, smooth surfaced endoplasmic reticulum (sER) (Fig. 1) is abundant except in perinuclear area in which rough surfaced endoplasmic reticulum (rER) relatively well developed as small sacks or small flattened sacks. Mainly sER is generally recognized as vesicles, however occasionally it appeared as membrane whorls (Fig. 1) or cluster of vesicles which possess sometimes electron dense material in its center.

In area in which sER is abundant, a few number of double walled tubules (Fig. 2) are rarely observed. The outer membranes of this structure are occasionally continuous with neighbouring vesicular sER.

Osmiophilic dense bodies appeared in cytoplasm are divided morphologically into two types. Dense bodies of the first type are generally round in shape and bounded by a thin membrane. The second type of dense bodies shows irregular outline, and commonly appears in some areas of the cytoplasm in which sER is predominant and occasionally they are wrapped in several layers of flattened sacks of sER.

The Golgi apparatus recognized generally in perinuclear area is poorly developed. In the Golgi region, cytoplasmic inclusions corresponding to secretory granules are not present.

Testicular Interstitial Cells Treated with Vitamin E (Fig. 4, 5, 6, 7, 8)

In comparison with normal interstitial cells, they are hypertrophied and mainly oval in shape, and touched closely to adjacent cells.

As compared with normal testicular interstitial cells, marked development of sER (Fig. 5) is observed within them and it occupies the greater part of cytoplasm and especially sER observed as a membrane whorl (Fig. 6, 7, 8) or cluster of vesicles is increased in size and number. Between membrane whorls consisted of only membranous component (Fig. 7) and the structures with an electron dense core (Fig. 8), transitional figures are often observed.

The irregular osmiophilic dense bodies (Fig. 6) are slightly increased in size and number and occasionally their profiles contain many vesicles.

Concerning the Golgi apparatus, no differences are recognized between the normal and the treated.

Discussion

In interstitial cells of normal mice, sER is well developed as Christensen & Fawcett ('66) noted in their work and moreover this development of sER is recognized also in interstitial cells of other species by several authors (Christensen & Fawcett, '61; Porter & Bonneville, '64; Christensen, '65). Especially sER observed as membranous whorl, which was first reported by Carr & Carr ('62), is also found in the mouse.

The testicular interstitial cells are considered as the main source of steroid hormones produced in testes as clearly confirmed by histochemical studies (Baillie & Griffiths, '64; Maeir, '65). Furthermore by recent biochemical studies (Toren, et al., '64; Shikita, et al., '64; Lynn & Brown, '58; Horfmann, '62), testosterone is biosynthesized from cholesterol and the enzymes mediating the synthesis of testosterone from pregnenolone is contained in microsomal fraction and further enzymes involved in biosynthesis of cholesterol are present in microsomal fraction too (Ying, et al., '65), that is, sER of testicular interstitial cells is the principal site of these enzymes. Fawcett ('65) suggested that membranes of sER may play the role of reservoir of cholesterol.

In testes of mice treated with vitamin E, interstitial cells are hypertrophied and their sER is markedly developed in comparison with the normal. From studies described above, these changes may mean that the interstitial cells are hyperfunctional in steroid hormone production.

Concerning membrane whorls, they are more abundant in vitamin E administered mice in comparison with the normal and moreover transitional figures are often observed between the structure consisted of membranous component and that with electron dense material.

These figures may possibly be interpreted as the results of the requirements of much cholesterol and thereafter the releasing of own cholesterol of the sER in hyperfunctional state. These ultrastructural changes of testicular interstitial cells of the mouse administered with vitamin E, which suggest an increased steroid hormone production, are well consistent with reports of Yamada & Ukei ('57) and Ichihara ('66) that administration of vitamin E activates the function of LH secretion of mouse anterior pituitary.

Summary

10 male mice of 14 weeks of age were treated daily with 10 mg vitamin E injection for 20 days. After the treatment, they were decapitated and their testes were examined by electron microscope.

- 1) By administration of vitamin E, testicular interstitial cells are enlarged and the sER is markedly developed, especially the membranous whorls are more abundant in comparison with normal interstitial cells.
- 2) From these data, it is conceivable that vitamin E administration activates the function of steroid hormone production in the testicular interstitial cells.

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Explanation of Figures

- Fig. 1. Low power electron micrograph of a mouse interstitial cell (Isc) lying near seminiferous tubules (sem). In cytoplasm, sER is well developed as small vesicle and membrane whorl (arrow). $\times 3000$
- Fig. 2. Higher magnification of left upper part of Fig. 1. A several number of double walled tubules (dwt) are present. At the end of them, outer membranes are continuous with vesicle of sER. Mitochondria (Mt) are seen and their internal matrices exhibit abundant tubular cristae. $\times 10000$
- Fig. 3. Higher magnification of an interstitial cell. In middle upper part, the hollowed cell surface (arrow) is studded with numerous microvilli (mv). Mitochondria (Mt), sER and free ribosomes are seen. $\times 10000$
- Fig. 4. Testicular interstitial cells (Isc) of the mouse treated with vitamin E. These cells are rounded in shape and enlarged in comparison with the normal. The sER is well developed in the greater part of cytoplasm. Membrane whorl (W) is clearly recognized. Irregular dense bodies are increased and scattered throughout the cytoplasm (arrow). $\times 3000$
- Fig. 5. Enlargement of Fig. 4. A portion of cytoplasm is filled with vesicle-like sER. Mitochondria in which internal matrices lost its electron density are found. At left upper corner microvilli (mv) are seen. $\times 10000$
- Fig. 6. Higher magnification of an interstitial cell. Abundant vesicle-like sER are observed. Irregular dense bodies (arrow) contain vesicles. Two membrane whorls (W) of relatively small size are seen and one of them possess irregular dense body in its central region. $\times 10000$
- Fig. 7. This structure exhibits concentric lamellae of tubular sER, however, at its central region it tends to exhibit amorphous material of low electron density. $\times 10000$
- Fig. 8. An irregular dense body is surrounded by concentric lamelle of tubular sER. $\times 10000$