Effects of Vitamin E Deficiency and Non-Biological Antioxidant (DPPD) on the Function of the Pituitary-Gonadal Axis of the Rat

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Summary The effects of vitamin E deficiency on pituitary-gonadal function in rats and the preventive effects of N,N'-diphenyl-p-phenylene diamine (DPPD) administration were examined by measuring levels of pituitary and plasma follicle-stimulating hormone (FSH) and luteinizing hormone (LH), serum and testicular levels of testosterone, and affinity and receptor sites of FSH and LH in the testis by radioimmunoassay, at 180 days after feeding of a vitamin E-deficient diet and DPPD-administered diet. Light and electron microscopic examinations were also performed on the pituitary gland and testis.

In the vitamin E-deficient rats, serum and liver α-tocopherol concentrations decreased significantly and erythrocyte hemolytic rate and serum and tissue malondialdehyde levels increased significantly. However, the increase of hemolytic rate and malondialdehyde concentration in the vitamin E-deficient rats was somewhat lessened by the administration of DPPD.

In the vitamin E-deficient rats, the gonadotropic cells in the pituitary gland manifested accelerated secretory function indicated by enlargement of cells, development of Golgi apparatus and accumulation of secretory granules, while FSH and LH concentrations in the pituitary and serum were not affected by vitamin E deficiency. However, the testosterone concentrations in the plasma and testis were significantly lower in the vitamin E-deficient rats. The decrease of testosterone in plasma and tissue was prevented by the administration of DPPD, while the degeneration of seminiferous tubules was not completely restored by DPPD. It is concluded that DPPD can compensate to some degree for the lack of
antioxidative activity due to vitamin E deficiency.

**Key Words** vitamin E deficiency, antioxidant DPPD, FSH- and LH-receptor, pituitary-gonadal function

The degeneration of the testis of rats is one of the classic symptoms of vitamin E deficiency and has been well documented since the pioneer work of Mason (1,2). The vitamin E-deficient animals show extensive irreversible degeneration of the seminiferous tubules, while the interstitial cells and accessory sex glands are apparently not affected. A marked increase in the number and size of basophilis was also observed in the anterior pituitary of vitamin E-deficient rats (3–5). An electron microscopic study of the anterior pituitary of vitamin E-deficient rats showed accelerated secretory activity of the gonadotrophs indicated by the development of Golgi apparatus and cytoplasmic vacuoles and accumulation of secretory granules (6,7).

However, very little attention has been paid to hormone status in vitamin E-deficient animals. FSH and LH concentrations in the serum and pituitary gland of vitamin E-deficient rats were measured by radioimmunoassay by Umeda et al. (8) and Akazawa (7). Umeda et al. (8) reported that FSH and LH concentrations in the pituitary and plasma were significantly lower in vitamin E-deficient rats than those in the controls, but testosterone concentrations in the testis and plasma were not affected by vitamin E deficiency. However, in our previous studies (6,7), the pituitary FSH content rose significantly over 90 days feeding of a vitamin E-deficient diet and the mean levels of pituitary LH and of serum FSH and LH also increased (but not significantly) at 60 and 90 days feeding on the same diet.

On the other hand, vitamin E has been regarded as important for its antioxidative action. In particular, it inhibits the peroxidation of polyunsaturated fatty acids which are the main components of the intercellular biomembrane. It is well known that vitamin E is a biological antioxidant to prevent excessive peroxidation, which may affect the maintenance of the function of hormone production in the pituitary-gonadal axis. On the other hand, a non-biological antioxidant, DPPD, has been reported to inhibit the increased hemolysis in animals fed on retinol- or vitamin E-deficient diet (9,10). Therefore, DPPD may have some effect in the prevention of lesions induced by vitamin E deficiency.

In the present study, biochemical and histological investigations were performed on male rats fed on vitamin E-deficient diet and on control rats given vitamin E or DPPD supplemented diet, to examine the role of vitamin E in the pituitary-gonadal axis. The concentration of FSH, LH and testosterone in the plasma and tissue, receptor sites and binding affinities of FSH and LH in the testis, and the histological changes of pituitary and testis were investigated in male rats deficient in vitamin E and in control rats.
MATERIALS AND METHODS

Animals. Forty weanling male Wistar rats were divided into four dietary groups and fed for 180 days either on a vitamin E-deficient or control (dl-α-tocopherol acetate, 10 mg/100 g diet, supplemented) diet. Groups 1 and 3 were fed on the control diet and groups 2 and 4 on a vitamin E-deficient diet. After 65 days of feeding on the experimental diets, groups 3 and 4 were further fed on a diet with 0.03% DPPD added.

Diets. The composition of the vitamin E-deficient diet for animals was as follows: Corn starch 34% (in w/w), α-wheat starch 10%, vitamin-free casein 25%, powdered filter paper 8%, granulated sugar 5%, mineral salts (Harper salt) 6%, vitamin mixture (except E) 2%, corn oil 10%. The vitamin mixture consisted of vitamin A acetate 1,000 IU, D3 200 IU, B6 8.0 mg, B12 0.001 mg, C 60 mg, K3 10.4 mg, biotin 0.04 mg, thiamin HCl 2.4 mg, folic acid 0.4 mg, Ca-pantothenate 10.0 mg, PABA 10.0 mg, niacin 12.0 mg, inositol 12.0 mg, and choline-Cl 4.0 mg.

Assays. 1) Measurement of the hemolytic rate was performed by Friedman's method using dialuric acid.
2) Vitamin E (α-tocopherol) levels in the plasma and liver were determined by the fluorometric method of Katsui (11).
3) Malondialdehyde levels in the serum and tissues were determined by the method of Yagi (12) using thiobarbituric acid.
4) FSH and LH concentrations in the pituitary and serum were determined by radioimmunoassay using the NIADDK kits (NIH, Bethesda, Md., USA). The pituitary homogenate was diluted 100 times with 0.1 M phosphate buffer saline (pH 7.4) before assay.
5) Testosterone concentration in the testis and plasma was determined by radioimmunoassay using 125I testosterone radioimmunoassay kit (Eiken Co.).
6) Receptor site and affinity for FSH and LH in the testis: All the testes of the same group of animals were pooled, weighed and homogenized in a teflon-glass homogenizer with 1 ml/testis of 50 mM Tris-HCl buffer solution containing 0.2 M sucrose. The homogenates were centrifuged at 15,000 rpm for 20 min in a refrigerated centrifuge. The precipitates were washed with 50 mM Tris-HCl buffer and used as receptor preparations. Protein concentrations in the suspensions were determined by the method of Lowry et al. (13). Bindings of radioligands to the FSH and LH receptors were performed in 0.5 ml of Tris-HCl buffer (pH 7.5) containing 5 mM MgCl2 and 0.1% bovine serum albumin (14, 15), employing 1.2 mg of protein of the testicular homogenates, FSH (NIAMDD I-3) and hCG (CR119) radioiodinated with the lactoperoxidase method (16). Incubation of radioiodinated FSH and hCG (0.5–1 ng) was carried out with increasing concentrations of the unlabeled hormones. Non-specific bindings were determined by incubation of the homogenates with the respective tracers in the presence of 50 IU of PMG (pregnant mare serum gonadotropin) or hCG.

Scatcherd plot analyses were carried out as follows: Bound/free ratios were determined.
plotted against bound amounts and a first order regression line was calculated. The association constant was obtained from the slope of the line, and the receptor site was calculated from the X-intercept.

**Histological examinations.** Half of the anterior pituitary and the testis were fixed separately in an ice-cold (2°C) solution of formaldehyde-glutaraldehyde fixative buffered at pH 7.2 for 2 h according to the method of Karnovsky (17). The tissues were then transferred to the 0.1 M phosphate buffer solution for 24 h, post-fixed in the phosphate-buffered 1% osmium tetroxide solution for 2 h, dehydrated, and embedded in an Epon mixture. Ultrathin sections were cut with glass knives on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate followed by lead citrate, and examined with a Hitachi HS-7 electron microscope. Semi-thin sections were also cut, stained with toluidine blue and examined with a light microscope. The other half of the testis was fixed in Bouin’s solution and embedded in paraffin for light microscopic observations.

**RESULTS**

1. **Body growth and weight of endocrine organs**

The ratios of the final weights of endocrine organs to body weights in the four groups of rats are shown in Table 1. Body weight did not differ significantly among the four dietary groups. The weights of hypophysis, testis, adrenals and thyroids did not show any significant change among the four groups. The tissue weights of prostate and seminal vesicles decreased significantly in vitamin E-deficient group 2 compared to the control groups 1 and 3.

2. **Erythrocyte hemolytic rate and vitamin E concentration**

The serum and liver α-tocopherol concentration and erythrocyte hemolytic rate of the four experimental groups are shown in Table 2. There were significant decreases in vitamin E concentration in groups 2 and 4, which were fed on the vitamin E-deficient diet.

The erythrocyte hemolytic rate was always very low in the vitamin E-supplemented rats of groups 1 and 3, while it was extremely high, at more than 98% in the vitamin E-deficient groups 2 and 4. However, in group 4, which was fed on the vitamin E-deficient diet with DPPD added, the hemolytic rates at 80 and 111 days after feeding were 16.9% and 52.1%, respectively. Thus, the administration of DPPD somewhat restored the hemolysis induced by the vitamin E deficiency.

3. **Malondialdehyde concentration in the serum, testis and liver**

In control group 1, the value of serum malondialdehyde concentration increased slightly with age, while this increase was inhibited in group 3 which was fed on the diet containing vitamin E and DPPD. The value of serum malondialdehyde concentration increased in the vitamin E-deficient group 2 more than 2 times that of the control groups 1 and 3, while this increase was suppressed slightly.
Table 1. Effect of vitamin E deficiency and administration of DPPD on endocrine organs (mg/100 g body weight).

| Groups          | No. of rats | Body weight (g) | Hypophysis (mg) | Testis (mg) | Prostata (mg) | Seminal vesicle (mg) | Adrenals (mg) | Thyroids (mg) |
|-----------------|-------------|-----------------|-----------------|-------------|---------------|----------------------|---------------|--------------|
| 1 + VE          | 10          | 473.4           | 2.18            | 724         | 57.6          | 176.8                | 9.82          | 4.40         |
| 2 - VE          | 10          | 465.3           | 1.96            | 737         | 35.4*         | 111.6**              | 9.90          | 5.01*        |
| 3 + VE + DPPD   | 10          | 482.6           | 2.21            | 712         | 56.6          | 163.5                | 9.42          | 4.41         |
| 4 - VE + DPPD   | 10          | 505.0           | 1.95            | 690         | 45.2          | 153.0                | 8.67          | 4.47         |

* *p < 0.05, **p < 0.01.

Table 2. Effect of vitamin E deficiency and administration of DPPD on erythrocyte hemolysis and concentration of \( \alpha \)-tocopherol.

| Groups          | Erythrocyte hemolysis (%) | \( \alpha \)-Tocopherol concentration |
|-----------------|---------------------------|--------------------------------------|
|                 | Days | 44 | 145 (80)* | 176 (111) | Serum (mg/dl) | Liver (\( \mu \)g/g) |
|                 |      |    |           |           | 180 (115)     | 180 (115)         |
| 1 + VE          | 5.5 ± 0.6\( ^b \) | 0.6 ± 0.7 | 3.9 ± 5.0 | 0.79 ± 0.15  | 33.8 ± 10.0       |
| 2 - VE          | 98.7 ± 1.4 | 99.5 ± 1.1 | 98.6 ± 2.2 | 0.09 ± 0.03*** | 3.1 ± 1.0*** |
| 3 + VE + DPPD   | 5.9 ± 0.1  | 0.1 ± 0.1  | 1.4 ± 1.8  | 0.84 ± 0.12  | 47.3 ± 9.8         |
| 4 - VE + DPPD   | 99.5 ± 16.9 | 16.9 ± 12.0 | 52.1 ± 19.4 | 0.09 ± 0.02*** | 3.6 ± 2.8*** |

\( ^a \) = Days after administration of DPPD. \( ^b \) M ± SD. ***p < 0.001.
Table 3. Malondialdehyde concentration in serum, liver and testis.

| Days  | Serum (nmol/ml) | Testis (nmol/g) | Liver (nmol/g) |
|-------|----------------|----------------|---------------|
| 1 + VE | 2.78 ± 0.84b   | 3.57 ± 3.08    | 55.60 ± 23.67 |
| 2 - VE | 6.58 ± 1.74*** | 7.86 ± 3.52*   | 120.39 ± 25.57*** |
| 3 + VE + DPPD | 2.20 ± 0.49 | 2.04 ± 1.37 | 25.03 ± 6.29 |
| 4 - VE + DPPD | 7.18 ± 1.53*** | 5.65 ± 3.30 | 49.71 ± 11.68 |

*a( )=Days after administration of DPPD. b M ± SD. *p<0.05, **p<0.01, ***p<0.001.

Table 4. Serum and pituitary levels of LH and FSH in the vitamin E-deficient and DPPD-administered rats.

| Groups | LH<sup>b</sup> | FSH<sup>b</sup> |
|--------|----------------|----------------|
|        | Serum (ng × SI/ml) | Pituitary (µg × SI/pit) | Serum (µg × SI/ml) | Pituitary (µg × SI/pit) |
| 1 + VE  | 0.74 ± 0.30c | 9.70 ± 3.22 | 0.50 ± 0.08 | 220 ± 87 |
| 2 - VE  | 0.55 ± 0.17 | 10.39 ± 2.79 | 0.56 ± 0.06 | 299 ± 88 |
| 3 + VE + DPPD | 0.62 ± 0.43 | 10.98 ± 4.87 | 0.56 ± 0.18 | 219 ± 125 |
| 4 - VE + DPPD | 0.44 ± 0.13 | 11.32 ± 3.15 | 0.55 ± 0.09 | 273 ± 69 |

<sup>a</sup>LH = NIH-LH-SI. <sup>b</sup>FSH = NIH-FSH-SI. <sup>c</sup>M ± SD.

in the vitamin E-deficient group 4 administered DPPD (Table 3).

The values of malondialdehyde concentration in the testis and liver increased significantly in the vitamin E-deficient group 2, but did not increase in group 4 fed on the vitamin E-deficient and DPPD-added diet.

4. FSH and LH concentrations in the serum and pituitary

FSH and LH concentrations in the pituitary and serum, taken from the rats deficient in vitamin E for 180 days and from the rats administered DPPD for 115 days, are shown in Table 4. The serum LH concentration in vitamin E-deficient groups 2 and 4 was lower than that of the vitamin E-supplemented groups 1 and 3, while the LH concentration in the pituitary was slightly higher in the vitamin E-deficient groups 2 and 4 than that of the vitamin E-supplemented group 1. However, these differences were statistically not significant. The serum FSH concentra-
Table 5. Testosterone concentration in serum and testis.

| Groups | Serum (ng/dl) | Testis (ng/g) |
|--------|--------------|--------------|
| 1 + VE | 257.1 ± 83.8*| 51.9 ± 26.7  |
| 2 − VE | 147.4 ± 46.9**| 23.0 ± 5.3*  |
| 3 + DPPD| 235.4 ± 60.9 | 42.4 ± 8.5   |
| 4 − VE + DPPD| 284.0 ± 58.8 | 59.6 ± 26.8 |

*M ± SD. *p < 0.05, **p < 0.01, compared with group 1.

...tions of the four groups did not show any remarkable difference, while the FSH concentrations in the pituitary were slightly higher (statistically not significant) in the vitamin E-deficient groups 2 and 4 than those in the vitamin E-supplemented groups 1 and 3.

5. Testosterone concentrations in the serum and testis

Testosterone concentrations in the testis and serum, taken from the rats deficient in vitamin E for 180 days and from the rats administered DPPD for 115 days, are shown in Table 5. They decreased significantly in the vitamin E-deficient group 2 compared with those of the vitamin E-supplemented control group 1. However, in the vitamin E-deficient group 4 administered DPPD, they did not differ from those in the vitamin E-supplemented control groups 1 and 3. This means that the administration of DPPD prevented the depression of testosterone concentration induced by the vitamin E deficiency.

6. Receptor site and affinity of FSH and LH in the testis

The number of FSH receptor sites in the testis increased slightly but the affinity of FSH decreased in the vitamin E-deficient group 2 compared with vitamin E-supplemented control group 1 (Table 6). These effects in the vitamin E-deficient rats were prevented by the administration of DPPD (group 4). The number of receptor sites and affinity of LH in the testis did not differ significantly among the four groups.

7. Histological examinations of the pituitary and testis

In the vitamin E-deficient rats, pituitary gonadotrophs increased markedly in number and size, some of them being 4 or 5 times the size of the controls (Figs. 1, 2, 7 and 8). These hypertrophic gonadotrophs were oval or polygonal in shape and contained an eccentrically located nucleus. They also had well developed Golgi apparatus, well developed endoplasmic reticulum with dilated cisternae and numerous secretory granules 170 to 300 nm in diameter (Fig. 8). Some of the gonadotrophs appeared to be signet ring cells containing large vacuoles developed...
Table 6. Receptor site and affinity.

| Groups       | LH receptors        | FSH receptors       |
|--------------|---------------------|---------------------|
|              | Site (fmol/mg)      | $K_a$ (10^9 M^{-1}) | Site (fmol/mg) | $K_a$ (10^9 M^{-1}) |
| 1 + VE       | 12.14 ± 1.54a       | 39.67 ± 5.83        | 7.04 ± 0.32    | 7.89 ± 0.83        |
| 2 - VE       | 11.38 ± 0.40        | 39.03 ± 8.58        | 9.38 ± 1.17    | 4.69 ± 0.51        |
| 3 + VE + DPPD| 8.83 ± 0.22         | 50.67 ± 7.13        | 6.57 ± 1.19    | 6.53 ± 0.86        |
| 4 - VE + DPPD| 10.54 ± 0.36        | 44.92 ± 8.16        | 7.42 ± 0.35    | 7.68 ± 0.75        |

*a M ± SD.  $K_a$, association constant.

by fusion of enlarged cisternae of the endoplasmic reticulum. The cells of the anterior pituitary of vitamin E-deficient and DPPD-administered rats (group 4) had a normal appearance (Fig. 3).

No significant differences were observed in weight of testis between the vitamin E-deficient (groups 2 and 4) rats and controls (groups 1 and 3). However, some of the vitamin E-deficient rats showed atrophic seminiferous tubules and degeneration of spermatocytes (Fig. 5). The seminiferous tubules of these testes were comprised largely of Sertoli cells and a few spermatogonia (Fig. 9). The tubules contained a fibrous formation in the lumen, the former consisting of degenerated cytoplasm of Sertoli cells. The interstitial cells also seemed to decrease in number and size.

Administration of DPPD in the vitamin E-deficient rats did not lead to complete recovery from the degenerative changes in the seminiferous tubules of the vitamin E-deficient rats (Fig. 6). Spermatocytes of some seminiferous tubules degenerated and fell off into the lumen of the tubules (Fig. 10).

DISCUSSION

Erythrocyte hemolytic rate and malondialdehyde levels in the serum and

Fig. 1. Anterior pituitary cells of vitamin E-supplemented control rat, showing normal appearance of pituitary cells. × 400.
Fig. 2. Anterior pituitary cells of vitamin E-deficient rat (2–3), showing enlarged gonadotrophs (clear cells). × 400.
Fig. 3. Anterior pituitary cells of vitamin E-deficient and DPPD-administered rat (4–3), showing nearly normal appearance. × 400.
Fig. 4. Seminiferous tubules of vitamin E-supplemented control rat, showing normal appearance of tubules. × 200.
Fig. 5. Degenerated seminiferous tubules of vitamin E-deficient rat (2–3). × 200.
Fig. 6. Seminiferous tubules of vitamin E-deficient and DPPD-administered rat, showing spermatocytes fallen off into the lumen. × 200.

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Fig. 7. Electronmicrograph of the anterior pituitary cells of control rat, showing normal appearance of gonadotroph (GT). ×3,000.

Fig. 8. Electronmicrograph of hypertrophied gonadotroph (GT) in the anterior pituitary of vitamin E-deficient rat (2–3). ×3,000.

Fig. 9. Electronmicrograph of seminiferous tubules of vitamin E-deficient rat (2–3), showing degenerated spermatogonium (G) and Sertoli cells (S). ×3,000.

Fig. 10. Electronmicrograph of testicular interstitial cells (IC) of vitamin E-deficient and DPPD-administered rat. ×3,000.
tissues increased remarkably in the vitamin E-deficient group 2, but these increases were somewhat prevented by administration of DPPD (group 4). Erythrocyte hemolytic rate is well correlated inversely to the \( \alpha \)-tocopherol concentration in the serum and tissues. However, when DPPD was administered to vitamin E-deficient rats, the erythrocyte hemolytic rate was slightly inhibited as compared with that of vitamin E-deficient group 2, but \( \alpha \)-tocopherol concentration in the serum and liver was not different from group 2. Kumar et al. (10) reported that \( \alpha \)-tocopherol and another lipid antioxidant, DPPD, inhibited the hemolysis of cells from retinol-fed rats, and Moore and Sharman (9) have also shown that DPPD feeding can correct the abnormal hemolysis induced by vitamin E deficiency. From these results, it is considered that the hemolytic rate may be affected by the antioxidative activity of vitamin E.

It is well known that vitamin E deficiency causes the increase of lipid peroxides as estimated by malondialdehyde concentrations in the serum and tissues. In this experiment, the administration of DPPD to the vitamin E-deficient rats prevented an increase of malondialdehyde concentration in the serum and tissues of vitamin E-deficient rats. Therefore, it is considered that the increase of malondialdehyde concentration may be mainly induced by the lack of antioxidative activity of vitamin E in the deficient rats.

In vitamin E-deficient rats, LH concentrations in the pituitary and serum did not change significantly, but the concentration of FSH in the pituitary was slightly (statistically not significant) higher than that of the control. Testosterone concentrations in the serum and testis were significantly lower than those in control rats. In our previous studies (Akazawa, 6,7), it was shown that the pituitary FSH concentration rose significantly after 90 days of vitamin E deficiency and that the mean concentrations of pituitary LH and of serum FSH and LH also increased but that it was statistically not significant. However, Umeda et al. (8) reported that FSH and LH concentrations in the pituitary and serum were significantly lower in rats after 7 months of vitamin E deficiency as compared to the controls. Must et al. (18) reported that serum FSH and LH concentrations rose in \( \text{H}^{re/+/} \) rats, which developed seminiferous tubule failure. FSH concentrations rose in \( \text{H}^{re/+/} \) rats to 140–170\% of those of normal male littermates after 30 days of age, while LH concentrations also rose in \( \text{H}^{re/+/} \) rats to 3 times normal by 240 days of age. The differences in the data on gonadotropin levels in vitamin E-deficient rats may depend on the duration of vitamin E deficiency, the degree of degeneration of testis and concentrations of gonadal steroid hormones in the blood.

The significant decrease of the plasma testosterone concentration in the vitamin E-deficient rats in the present study is in good agreement with the results of Lees et al. (19), who reported that the plasma testosterone concentrations in male rats given a vitamin E-deficient diet for 130 days were significantly lower than those in rats given the diet supplemented with vitamin E. Barnes and Smith (20,21) found decreased activity of some of the enzymes involved in steroid hormone synthesis in the adrenal and testis taken from rats deficient in vitamin E from weaning to a
maximum of 291 days. Lees et al. (19) assumed that these changes in vitamin E-deficient rats could be brought about by reduced production of pituitary hormones, reduced response of the target tissue to the tropic hormones, or by some more fundamental biochemical changes in the cells resulting in reduced production of steroid hormones.

In this paper, the receptor site and affinity of FSH and LH in the testis were investigated in relation to the function of vitamin E which contributes to the stabilization of the plasma membrane holding receptor. The receptor site and binding affinity of LH in the testis did not differ among the four groups, but the binding affinity of FSH decreased only in vitamin E-deficient group 2. The decrease of affinity of FSH in the vitamin E-deficient rats was prevented by the administration of DPPD.

The decrease of the serum and testicular concentrations of testosterone in the vitamin E-deficient rats was prevented by administration of antioxidant, DPPD. Therefore, the depression of testosterone in the vitamin E-deficient rats may be caused mainly by the lack of antioxidative activity in the tissue. Kitabchi et al. (22) have reported that treatment with an antioxidant, BTH (butylate hydroxytoluene) does not affect lipid peroxidation or steroidogenesis in the adrenal cells of vitamin E-deficient rats. However, Krishnamurthy and Bieri (23) suggested that the variation among antioxidants in their ability to substitute for vitamin E is due primarily to differences in their availability to the body and subsequent deposition in the tissue. Chan et al. (24) described that DPPD prevented the development of muscular dystrophy induced by vitamin E deficiency in spite of very low plasma α-tocopherol concentration. King (25) reported that DPPD administration prevented testicular degeneration induced by vitamin E deficiency. In the present study, however, the degeneration of seminiferous tubules in the vitamin E-deficient rats was not completely reversed by the administration of DPPD, but the interstitial cells showed more normal features in the DPPD-administered rats than in the vitamin E-deficient rats.

The results of our study indicate that gonadal function was impaired by vitamin E deficiency, but that nevertheless the function of gonadotropin secretion by the pituitary did not change remarkably. Therefore, it is considered that vitamin E deficiency directly suppresses gonadal function but not pituitary function.

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REFERENCES

1) Mason, K. E. (1926): Testicular degeneration in albino rat fed a purified food ration. J. Exp. Zool., 45, 159–229.

J. Nutr. Sci. Vitaminol.
2) Mason, K. E. (1939): Differences in testis injury and repair after vitamin A-deficiency, vitamin E-deficiency and inanition. Am. J. Anat., 52, 153–239.
3) Nelson, W. O. (1933): Studies on the anterior hypophysis. III. The anterior hypophysis in vitamin E-deficient rat. Anat. Rec., 56, 241–259.
4) Koneff, A. A. (1939): Pituitary changes in male rats reared and maintained on 'pure' diets with and without vitamin E. Anat. Rec., 74, 383–399.
5) Ichihara, I. (1969): Electron microscopic studies of anterior pituitary glands of vitamin E-deficient male mice. J. Anat., 104, 455–465.
6) Akazawa, N. (1977): Electron microscopic studies on the pituitary and other endocrine organs in vitamin E deficient rat. Vitamin (Vitamins) (J. Vitamin Soc. Jpn.), 51, 141–151.
7) Akazawa, N. (1978): The effect of vitamin E deficiency on the function of pituitary-gonadal system. Vitamin (Vitamins) (J. Vitamin Soc. Jpn.), 52, 271–277.
8) Umeda, F., Kato, K., Muta, K., and Ibayashi, H. (1982): Effect of vitamin E on function of pituitary-gonadal axis in male rats and human subjects. Endocrinol. Jpn., 29, 287–292.
9) Moore, T., and Sharman, I. M. (1961): Prevention of the injurious effects of excessive cod liver oil by its fortification with vitamin E. Br. J. Nutr., 15, 297–303.
10) Kumar, P. S., George, T., Jayanthi Bai, N., and Krishnamurthy, S. (1979): Effect of lipid antioxidants on rat erythrocyte hemolysis. Int. J. Vitam. Nutr. Res., 49, 352–358.
11) Katsui, G. (1980): Assay methods of vitamin E. 2. Fluorometric method. Vitamin (Vitamins) (J. Vitamin Soc. Jpn.), 54, 449–453.
12) Yagi, K. (1975): Micro-determination of lipoperoxide in blood plasma or serum. Vitamin (Vitamins) (J. Vitamin Soc. Jpn.), 49, 403–405.
13) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem., 193, 265–275.
14) Dufau, M. L., Podesta, E. J., and Catt, K. J. (1975): Physical characteristics of the gonadotropin receptor-hormone complexes formed in vivo and in vitro. Proc. Natl. Acad. Sci. USA, 72, 1272–1275.
15) Wakabayashi, K., Minegishi, T., Yorozu, Y., Igarashi, M., and Ichinohe, K. (1980): A sensitive radioreceptor assay for follicle stimulating hormone with PMS-primed immature rat ovary. Endocrinol. Jpn., 27, 87–93.
16) Miyachi, Y., Vaitukaitis, J. L., Nieschlag, E., and Lipsett, M. P. (1972): Enzymatic radioiodination of gonadotropins. J. Clin. Endocrinol. Metab., 34, 23–28.
17) Karnovsky, M. J. (1965): A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol., 27, 137 A.
18) Must, N. A., Santen, R. J., Huckins, C., and Bardin, W. (1978): Abnormalities of the pituitary-gonadal axis of H*e rats: A study of animals with an inherited disorder of seminiferous tubular and Leydig cell function. Biol. Reprod., 19, 797–806.
19) Lees, D., Barnes, M. McC., and Cox, J. E. (1982): Testosterone and corticosterone concentrations in the plasma of rats deficient in vitamin E. J. Reprod. Fert., 66, 543–545.
20) Barnes, M. McC., and Smith, A. J. (1975): The effect of vitamin E deficiency on androgen and corticosterone synthesis. Int. J. Vitam. Nutr. Res., 45, 342–348.
21) Barnes, M. McC., and Smith, A. J. (1975): The effects of vitamin E deficiency on some enzymes of steroid hormone biosynthesis. Int. J. Vitam. Nutr. Res., 45, 369–403.
22) Kitabchi, A. E., Nathans, A. H., and Kitchell, C. L. (1973): Adrenal gland in vitamin E deficiency. III. Inhibition of adrenocorticotropic hormone-induced steroidogenesis in
isolated adrenal cells by ascorbic acid. *J. Biol. Chem.*, 248, 835–840.

23) Krishnamurthy, S., and Bieri, J. G. (1962): Dietary antioxidant as related to vitamin E function. *J. Nutr.*, 77, 245–252.

24) Chan, A. C., Pritchard, E. T., and Choy, P. C. (1983): Differential effects of dietary vitamin E and antioxidants on eicosanoid synthesis in young rabbits. *J. Nutr.*, 113, 813–819.

25) King, D. W. (1964): Comparative effects of certain antioxidants on gestational performance and teratogeny in vitamin E deficient rats. *J. Nutr.*, 83, 123–132.