Effects of water-soluble vitamin E administration on the pituitary-gonadal system of the mouse

by

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Introduction

In vitamin E-deficient rat, it has been reported by many authors that vitamin E-deficiency induced a testicular atrophy and tubular degeneration, but the interstitial tissue was almost not affected, and moreover, it has been pointed out that this testicular degeneration in E-deficient rat was irreparable in nature while the accessory sex organs were almost not influenced by E-deficiency (see Mason's review, '39). But reports on E-deficient mouse have not so much been published: Beard ('26) reported that E-deficient mouse is almost same in testicular alterations as E-deficient rat, and Horikawa ('58) recognized a delayed spermatozoa without testicular degeneration, while Bryan and Mason ('40) and Goettsch ('42) observed no histological change in it after long experimental period. In E-deficient female animal, it is the agreed opinion among many investigators that the pregnancy was interrupted by the death and the resorption of the fetus (see Mason's review, '39), and abnormality of estrus cycle and ovarian dysfunctions are reported (Blumberg, '35; Martin and Moore, '39; Schneider, '39; Sasaki, '60).

In anterior pituitary of male rat, it has been observed that E-deficiency caused a marked increase in number and size of basophiles and a occurrence of “signet ring form cells” (Van Wagener, '25; Nelson, '32, '33; Müller and Müller, '37; Koneff, '39; Biddulph and Meyer, '41; Griesbach, Bell and Livingstone, '57), whereas in female rat pituitary, it is not agreed by the investigators whether E-deficiency caused histological change or not (Nelson, '33; Barrie, '37; Müller and Müller, '37).

On the other hand, it is said that overdoses of vitamin E caused an activation of spermatogenesis, but it is not distinct that over-
doses of vitamin E influenced on the ovarian functions. Recently, in our laboratory, effects of E-administration on anterior pituitary and testis of rat and mouse have been investigated (Yamada and Ukei, '57; Sakakura, '60; Torii, '60; Fujibayashi, '60; Okumura, '60; Fujino and Yoshioka, '62). However, in these experiments using fat-soluble vitamin E, it was often seen that vitamin E was still remained in subcutaneous region after the injection. The present study was undertaken by using easily absorbable water-soluble vitamin E for investigation of the effects of vitamin E on the pituitary-gonadal system.

Materials and Methods

In this study, 34 immature and 32 mature mice of both sex daily injected with water-soluble vitamin E (E-236-001 prepared by Eisai Co., Ltd.). Immature mice were injected with 10 mg from 23 days of age (immature group), and mature mice were injected with 20 mg from about 90 days of age (mature group), for periods of 5, 10, 15 and 20 days. At various stages, the animals were sacrificed with chloform anesthesia. As injection control animals, 16 immature and 16 mature mice of both sex were injected with each 0.2 cc and 0.4 cc of solvent of this preparation. Sixteen immature and 16 mature mice were used as normal control animals. They were killed following the same schedule as in the experimental group. Gonads and pituitaries thus obtained were fixed with Zenker-formalin acetic acid solution and Helly's solution, and embedded in paraffin. Serial sections at 4 microns were stained with hematoxylin-eosin stain, Heidenhain's azan stain and the periodic acid-Schiff reaction.

Observations

I. Immature group

1) Anterior pituitary

Following 5 injections: Acidophiles are numerously observed throughout the anterior lobe. They are well granulated in general. Medium sized acidophiles are predominant. Acidophiles with a negative Golgi image, a perinuclear halo and a cytoplasmic basophilic substance are occasionally seen. Nuclei of them are round or oval in shape, and are light vesicular, and the mitotic figures are often seen. Small acidophiles are fully granulated and almost not contain
a negative Golgi image, a perinuclear halo and a cytoplasmic basophilic substance, and also their nuclei are darker than those of the other acidophiles.

Beta cells are scattered in small numbers in the central area of the anterior lobe. They are polygonal or irregular in shape and densely packed with fine PAS positive granules. Nuclei of them are round or oval in shape and contain abundant chromatin granules.

Delta cells are found in small numbers, and they are located in clusters in the anterior portion and are also scattered in the central area of the anterior lobe. They are round, oval or polygonal in shape and filled with somewhat coarse PAS positive granules in the cytoplasm. The cytoplasmic granulation of delta cells are somewhat fewer than in the controls. Generally, delta cells are slightly smaller in size than in the mature animals. In female pituitaries, delta cells contain more frequently hyaline vacuoles than in the controls.

Theta cells are rarely observed throughout female anterior lobe, while in male they are almost not found. They have an eccentrically situated nucleus, a cap-like structure consisted of many basophilic filaments and the juxta-nuclear region is weakly stainable with PAS reagent.

Chromophobe cells are found most numerously in clusters or scattering throughout the anterior lobe. They are smaller in size than chromophile cells. They show an indistinct cell boundary and contain a few flocculent or granular material in the cytoplasm. Nuclei of chromophobes are round or oval in shape and light vesicular. Mitotic figures in these cells are occasionally found.

Following 10 injections: Acidophiles do not differ in occurrence, size and granulation from the controls. In some cases, large sized ones indicate a slight decrease in number in comparison with the controls. Nuclei of the acidophiles are generally darker than in the controls. Beta cells do not differ in occurrence and cytological detail from those of the normal controls. In some cases, beta cells indicate a slight decrease in size and granulation in general. Delta cells scarcely differ in occurrence and size from those of the normal. Generally, densely granulated delta cells are somewhat numerously found than in the controls (Figs. 2, 3), while in female sparsely granulated large delta cells frequently seen. Theta cells do not differ in occurrence and cytological detail from those of the normal. Chromophobe cells do not indicate any histological change.

Following 15 injections: Acidophiles do not differ in occurrence,
size and granulation from those of the normal. Beta cells do not differ in occurrence and size from those of the normal. They are well granulated in general, while there are a few relatively large beta cells which are lightly granulated, and of which nuclei contain abundant chromatin particles. Delta cells do not differ in occurrence, size and granulation from those of the controls. But in some cases lightly granulated delta cells are more numerously found than in the controls. Theta and chromophobe cells do not indicate any change.

Following 20 injections: Acidophiles do not differ in occurrence and granulation from those of the normal controls. But in some cases large sized acidophiles are somewhat more numerously than in the controls, and frequently contain a negative Golgi image, a perinuclear halo and a cytoplasmic basophilic substance. Their nuclei are light vesicular and contain somewhat coarse chromatin particles. Beta cells do not differ in occurrence and granulation from those of the normal. They are almost same as the normal controls in size, although there are a few large cells. Delta cells do not indicate any remarkable change in occurrence. They show a slight decrease in granulation but their size are not so large. Nuclei of them are light vesicular. In female pituitaries, delta cells contain somewhat more frequently hyaline vacuoles in the cytoplasm than in the previous stage. Theta cells do not differ in occurrence from the normal. Chromophobe cells do not show any remarkable change.

2) Testis

Following 5 injections: Spermatogonia are round or oval in shape, and are found on or near the basement membrane. The nuclei are round or oval in shape, and there are 2 types: the one is small one having reticular heavily staining chromatin flakes and is superior in number to the other dust-like nucleus. The dust-like nuclei of the cells are fewer than in the controls. Mitotic figures in spermatogonia are occasionally seen. Primary spermatocytes are often seen. Small primary spermatocytes are round or oval in shape, and are often seen either on or near the basement membrane. They are more numerous than large primary spermatocytes as those of the normal controls. Their nucleus is round in shape and includes darkly staining chromatin particles and net at resting stage, and frequently shows mitotic figure. Large sized primary spermatocytes are round, oval or polygonal in shape, and found in the middle zone of the germinal epithelium. It has a large sized nucleus containing
large chromosomes in general. Secondary spermatocytes are round or oval in shape and occupy the middle zone of the germinal epithelium in small numbers. They are smaller and much more rarely seen than the primary. The nuclei are round shaped and contain frequently large chromosomes in mitotic division, and in resting stage they contain loose-reticular chromatin net. Their mitotic figures are frequently found than in the controls.

Spermatids which are the most numerous in all germinal cells consists of several layers in the inner zone of the germinal epithelium. They are round, oval or polygonal in shape, and small in size. The nucleus is round or oval in shape and light vesicular with a distinct nucleolus. The cells are more frequently found than in the controls. While, maturing sperms are rather fewer than in the controls. Maturing sperms are oval or polygonal in shape and are numerously found in the inner zone of the germinal epithelium. The nucleus is vesicular and oval shaped or condensed hook-shaped, and connects its posterior pole with the tail. Mature sperm has a small spindle-shaped head staining further densely and a long tail. They are numerously found in the inner layer in many seminiferous tubules. Free sperms are rare.

Sertoli cells are scattered in seminiferous tubules. They are very large slender triangular in shape and are perpendicular to the basement membrane. The cell is situated with its base on the basement membrane, and apex of the cell is toward the seminiferous lumen. The large oval nucleus is situated near the base of the cell containing few fine chromatin particles and a distinct nucleolus. The nucleolus consists of a chain of 3 small bodies, the one centrally lying and staining with hematoxylin is slightly larger in size than the others, and the other two are heavily stained by eosin. Their mitotic figures are rarely seen.

Interstitial cells are numerously found between seminiferous tubules in clusters. They are polygonal, oval or round in shape and somewhat large in size than in the controls. The nucleus is round or oval in shape and has coarse chromatin granules and one or two nucleoli. The mitotic figures are relatively often seen. Generally, their cytoplasm have frequently some vacuoles which are somewhat more numerously found than in the controls.

Following 10 injections: In spermatogonia, darkly staining nuclei are more often seen than dust-like nuclei as those of the controls. Primary spermatocytes are very frequently found, and their mitotic figures are more often encountered than in the con-
trols. Secondary spermatocytes are almost similar in occurrence and cytological detail to those of the controls. Mitotic figures in these cells are somewhat fewer than in the previous stage. Spermatids are slightly more numerously found than in the previous stage, but almost same as the normal. Maturing sperms are not so abundant, but are slightly more numerously than in the controls (Fig. 5). Mature sperms and Sertoli cells do not indicate any change. Interstitial cells do not differ in number and size from those of the controls. However, vacuolated interstitial cells and number of vacuoles in the cytoplasm are more numerously found than in the controls. Mitotic figures are occasionally seen.

Following 15 injections: Spermatogonia are almost same in number and cytological detail as those in the previous stage. Primary spermatocytes are numerously encountered as those of the controls, and also their mitotic figures are somewhat more often seen than in the controls. Secondary spermatocytes indicate no histological change. Spermatids are more abundant in occurrence than in the previous stage, but do not differ from those of the controls. Maturing sperms are very numerously found in comparison with those of the controls. Sperms are more numerously seen than in the controls. Sertoli cells do not show any change. Hypertrophic or vacuolated interstitial cells are more frequently found than in the controls.

Following 20 injections: Spermatogonia are almost similar in number and cytological detail to those in the previous stage. Primary spermatocytes are not so many found and are slightly fewer than in the controls. Mitotic figures are not so much seen as those of the controls. Secondary spermatocytes do not indicate any change. Spermatids are very numerous and occupy the large portion of the germinal epithelium as the normal. Maturing sperms and mature sperms increase in number in comparison with those of the controls. Sertoli cells are almost same in number and cytological detail as the normal. Interstitial cells indicate an increase in size and vacuolation of the cytoplasm comparing with those of the controls.

3) Ovary

Following 5 injections: Primary follicles are numerously seen in the superficial zone of the cortex but somewhat fewer than in the controls. The oocyte is small in size and contains a large light vesicular nucleus with a distinct nucleoli and is surrounded by several follicular cells. Follicular cells is flat in shape and contain
an oval shaped and vesicular nucleus with indistinct nucleolus. Mitotic figures are often seen in the follicular cells.

Growing follicles are often seen in the superficial and slightly deep zone of the cortex. The oocyte indicates further increase in size, and also the nucleus shows a slight increase in size compared with that in the primary follicle. It has a heavily eosinophilic, homogenous, PAS positive oolemma. The oocyte is around by stratified follicular cells which are loose in connection and have small space filled with eosinophilic material between them. The nucleus of follicular cell is small in size and light vesicular and mitotic figures are often seen in the follicular cells. The follicle is surrounded by theca folliculi which consists of connective tissue cells, and in some growing follicles the theca folliculi develops into two layers, theca interna and externa. The nuclei of them are chromatin-poor and often indicate mitotic division.

Grown vesicular follicles are found in small numbers, and occupy almost whole thickness of the cortex. Granulosa cells have large vesicular nuclei and are slightly larger in size than follicular cells and show distinct cell boundary. Mitotic figure is almost not found in them. The follicular cavity contains exudate. Oocytes are almost similar in cytological detail to those of the growing follicles. Involution is found numerously at the various stages of primary, growing and vesicular follicles.

Corpora lutea are sometimes encountered and occupy almost whole thickness of the cortex. The wall of fresh corpus luteum collapses. Granulosa cell has a rather small nucleus which contain somewhat coarse chromatin particles and distinct nucleolus. Follicular cavity is irregular in shape and contains exudate and histiocytes. In some cases, slightly advanced corpora lutea are rarely seen (Fig. 6), but in the controls are almost not found. Majority of these lutein cells are arranged in radial cords and have a round or oval chromatin-poor swollen nucleus with one or two distinct nucleoli. The cytoplasm of lutein cell contains some vacuoles. Lutein cells in the peripheral portion of the corpus luteum contain small round or oval nuclei in general, and the nuclei contain more abundant chromatin than in the other lutein cells.

Medullary cords are slender around the hilus. They consist of squamous epithelial cells, and contain many blood vessels. They do not indicate any remarkable change.

Following 10 injections: Primary follicles are found slightly more numerously than in the previous stage. They are almost same
in cytological detail as the controls. Grown follicles are surrounded by stratified follicular cells, and grown vesicular follicles are more numerously found than in the previous stage. But, they do not indicate any change in comparison with those in the controls. Involution in the primary and growing follicles is often seen especially in deep zone of the cortex and the hilus ovarii as that in the controls. Fresh corpora lutea are occasionally seen, and advanced corpora lutea are somewhat rarely found. They are almost same in occurrence and cytological detail as in the controls. Medulla ovarii shows no change.

Following 15 injections: Primary and growing follicles do not differ in number and cytological detail from the normal. Involution in the primary and growing follicles are somewhat fewer found than in the controls. Growing vesicular follicles are more numerously than in the previous stage, but they do not differ in cytological detail from those of the normal. Fresh corpora lutea are almost similar in number and cytological detail to those in the previous stage. Medulla do not show any change.

Following 20 injections: Follicles are almost same in number and cytological detail as those of the controls. Fresh corpora lutea are fewer than in the previous stage, while advanced corpora lutea are observed as frequently as those in the previous stage, and they do not show any histological change comparing with those of the controls. Medulla also indicate no change.

II. Mature group

1) Anterior pituitary

Following 5 injections: Acidophile cells do not differ in number, size and cytoplasmic granulation from those of the controls. Mitotic figures are rarely observed. Beta cells also do not show any marked change. Delta cells indicate a slight decrease in granulation, but in some cases indicate slight increase in cytoplasmic granulation and slight decrease in size. In female pituitaries, delta cells are lightly granulated and contain often some hyalin vacuoles in the cytoplasm. Theta cells and chromophobe cells do not indicate any change. Mitotic figures are occasionally observed in the chromophobes.

Following 10 injections: Acidophiles do not differ in occurrence, size and cytological detail from those of the normal. Beta cells show a normal appearance in cytological detail. Occasionally, they are densely granulated with a slightly dark nucleus. Delta cells
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do not differ in occurrence and size from those of the controls in general. Heavily granulated delta cells are somewhat more numerous found than in the controls. Theta cells are almost similar to the normal controls. Chromophobes do not show any marked change.

Following 15 injections: Acidophiles do not differ in occurrence and cytoplasmic granulation from those of the normal. Medium sized acidophiles are predominant, but in some cases small acidophiles with picnotic nuclei are more frequently observed than in the controls. Such a small acidophile cells rarely contains a negative Golgi image, perinuclear halo and cytoplasmic basophilic substance. Beta cells show no marked change. Delta cells do not differ in occurrence from those of the normal. They are somewhat larger in size than in the controls in general and indicate a slight decrease in cytoplasmic granulation in comparison with those of the controls (Fig. 8). Theta cells do not show any change. Chromophobes also indicate no change.

Following 20 injections: Acidophiles do not differ in number from those of the normal. Large sized ones are somewhat more frequently observed than in the controls. The large acidophiles are sparsely granulated in general and contain more frequently a negative Golgi image, a perinuclear halo and a cytoplasmic basophilic substance. Beta cells do not differ in size and cytological detail from the normal in general. But, in some cases heavily granulated beta cells are somewhat numerously found. Delta cells do not differ in number from those of the controls. Their cytoplasmic granulation indicates a slight decrease, while they show a slight increase in size in comparison with those of the controls. Theta cells show no marked change. Chromophobes are almost same as the previous stage in cytological detail.

2) Testis

Following 5 injections: In spermatogonia, heavily staining nuclei are predominant, and among them, mitotic figures are occasionally seen. But they do not show any remarkable change. Primary spermatocytes do not differ in number and cytological detail. Mitotic figures are often seen like those of the controls. Secondary spermatocytes do not show any change. Mitotic figures are somewhat fewer than in the controls. Spermatids are the most numerous in the germinal cells. However, they do not differ in number and cytological detail from those of the normal. Spermatozoa are found numerously in each tubules except a few tubules as
those of the controls. Mature sperms are more numerously en-
countered than in the immature animals. Free sperms are some-
times seen in seminiferous lumen. Sertoli cells do not differ in
number and cytological detail from those of the controls. The
mitotic figures are rarely seen in the interstitial cells. They have
frequently some vacuoles in the cytoplasm as those of the controls,
and some show hypertrophic cytoplasm.

Following 10 injections: Spermatogonia do not differ from
those of the normal. Primary spermatocytes do not indicate any
marked change. Secondary spermatocytes are found in small num-
bers without marked histological change. Spermatids do not show
any change. Maturing spermatozoa are relatively abundant but
rather fewer than in the controls. While, mature spermatozoa
indicate a slight increase in number in comparison with those of
the controls (Fig. 10). Sertoli cells do not show remarkable change.
Interstitial cells showed an increase in size and vacuolation in the
cytoplasm comparing with the controls.

Following 15 injections: Spermatogonia, primary and secondary
spermatocytes, spermatids and maturing sperms are almost same
in occurrence and cytological detail as the previous stage. Mature
sperms almost indicate no marked change, but in some cases semi-
ferous tubules containing sperms are slightly less found than in
the controls. Sertoli cells show no marked change. Interstitial
cells have more numerous vacuoles in the cytoplasm than in the
controls. The cytoplasm is not so hypertrophic in general.

Following 20 injections: Spermatogonia, primary and secondary
spermatocytes, spermatids and maturing sperms are almost similar
to the previous stage. Mature sperms are more often seen in com-
parison with the controls. Sertoli cells do not indicate any change.
Interstitial cells are somewhat larger in size than in the controls,
but almost not differ in vacuolation from those of the controls.

3) Ovary

Following 5 injections: Primary and growing follicles are re-
latively often seen in the superficial zone of the cortex as the normal.
In the follicular cells, mitotic figures are often seen. These follicles
do not differ in number from the normal. Involution is frequently
found in growing follicles, especially in those in the deep zone of
the cortex and the medulla. Growing vesicular follicles are not so
often found but almost similar in number and cytological detail to
those of the controls. Corpora lutea are more frequently found
than in the immature animals. Corpora lutea at advanced stage
are somewhat more frequently found than in the controls. Advanced corpora lutea consist of lutein cells with chromatin-poor nuclei. In medulla no histological change is seen.

Following 10 injections: Primary and growing follicles do not differ in occurrence and cytological detail from the controls. A number of corpora lutea are transformed into corpora fibrosa as the normal. Lutein cells which underwent hyaline vacuolation are invaded extending from center to periphery. Medulla do not indicate any change.

Following 15 injections: No significant change occurred in follicles and corpora lutea and fibrosa, but, in some cases atretic follicles are slightly more numerously found in deep zone of the cortex than in the controls (Fig. 12). Medulla do not indicate any change.

Following 20 injections: The ovary shows a normal appearance.

Discussion

a) Anterior pituitary

The studies on E-deficient anterior pituitary have been published numerously, but there were not so many reports on pituitary treated with overdoses of vitamin E. *Suardi* ('58) described that the rat hypophysis showed an increase of the number of basophilic cells, when at least 100 mg of vitamin E was given. In our laboratory, however, since several years, the pituitaries of vitamin E administered animals have been investigated histologically. *Yamada* and *Ukei* ('57) reported that overdoses of vitamin E caused an increase in occurrence, size, cytoplasmic granulation and vacuolation of delta cells in the rat anterior pituitary, and also *Nakamura* ('60) described almost similar histological findings in pregnant mouse anterior pituitary to prior study (Yamada and Ukei, '57). *Torii* ('60) observed that basophiles indicated an early development in E-administered immature mice. However, *Fujibayashi* ('60) and *Okumura* ('60) reported that in E administered mature mice, the anterior pituitary did not indicate marked histological changes.

In this experiment used water-soluble vitamin E, generally the histological changes were not so remarkable, but some changes were mostly found in delta cells. That is, delta cells showed generally an increase in number and a decrease in cytoplasmic granulation. Moreover, in this study, spermatocytes were activated and also interstitial cells indicated an increase in vacuolation and size. From
these findings, the author assumed that vitamin E influenced to the pituitary-gonadal system, although ovary indicates almost no marked histological change.

Acidophiles did not show marked change, but in some cases, large sized or small sized cells were somewhat frequently observed. However, as acidophiles were not only influenced by vitamin E but also various medicine, such changes are not always peculiar in the animals treated with vitamin E. Acidophiles are associated with secretion of growth hormone which has an intimate relationship with metabolism. Therefore, these findings may depend on the changes in metabolism occurring under the condition of vitamin E injection.

b) Testis

In vitamin E-deficient rat testis, many investigators observed testicular degeneration, but E-deficient mouse testes have been reported by only several authors; Beard ('26) described testicular degeneration as seen in rat testis, but Bryan and Mason ('40) and Goetttsch ('42) observed no histological change, and also Horikawa ('58) did not found testicular development in immature mouse. A few investigators have reported on the E-administered mouse and rat testis. Zondeck, Brzezinski and Sulman ('42) reported that the spermatogenesis of immature rat was not activated by vitamin E. On the other hand, Bottigioni and Sturani ('56) observed in rat testis that pharmacological doses of vitamin E showed a marked activation of the testicular function and the spermatogenesis, although it was not to same extent as observed after treatment with pharmacological doses. Fujino and Yoshioka ('62) found an increase in number of spermatozoa, and in size and vacuolation of interstitial cells in fat-soluble E administered mouse testis.

In this data, vitamin E showed an activation of the spermatogenesis. That is, immature mice, meiotic division and maturing and mature sperms were more frequently seen than in the controls, and interstitial cells indicated an increase in number of vacuoles within the cytoplasm. In mature group, activation of spermatogenesis was found scarcely, and further in some cases spermatogenesis was rather arrested, but interstitial cells showed generally an increase in size and vacuolation.

From these findings, it was thought that vitamin E activated the spermatogenesis and the function of interstitial cells. It might be thought that effected point of vitamin E was differed from those
of androgen, as Álvarez ('47) described that vitamin E reinforced the movement and life force of the spermatozoa in semen, but that the effect of androgen could not be neglected from the finding of interstitial cells in this experiment and the reports that testosterone reinforced the action of vitamin E (Adamstone, '41; De Watteville et al., '48).

c) Ovary

In female animals, it has been discussed the effect of vitamin E to sex development. Adler and Boltink ('29), Verzar ('29, '31, '32), Szarka ('30), Yonekawa ('38) and Sasaki ('60) described that vitamin E caused premature oestrus, early vaginal opening and uterine hypertrophy in premature female rat and mice. But, there published many reports which vitamin E did not effect for ovary in rat and mouse.

In this experiment, at 28 days of age following 5 injections, the ovary did not indicate marked change, but in some cases, corpora lutea were rarely seen, while in the controls they were almost not found. However, it could not be concluded that vitamin E caused early development of female sex organs because the author did not observe on the more younger animals. In immature group following 15 injections, involution of the follicles were somewhat less found than in the controls. Further, in mature mice, at early stage of experiment, advanced corpora lutea were somewhat more frequently found than in the controls, but at late stage, in some cases, atretic follicles are slightly more numerous than in the controls. It was reported that overdoses of vitamin E stimulated the sexual function (Evans and Burr, '27; Carbonini, '56), and that it gave no influence on the gonadal function, otherwise Sasaki ('60) described that, although large doses of vitamin E had no influence on the fertility of mature rat, the new-born infant died more frequently than those of the controls. From these data, although in some cases, atretic follicles were somewhat frequently observed, accurate conclusion could not be said.

d) Influence of vitamin E on pituitary-gonadal system

Many investigators have been reported that E-deficient rat pituitaries show histological changes almost similar to those of castrated animals as already wrote. Further, basophiles especially delta cells were influenced by overdoses of vitamin E (Yamada and Ukei, '57; Suardi, '58; Nakamura, '60; Torii, '60). In this data, delta cells indicated an increase in number or decrease in cytoplasmic granulation, and spermatogenesis of their testes were
more stimulated comparing with those of normal testis. From these findings, it is concluded that vitamin E influences on pituitary-gonadal system in mouse.

In vitamin E deficient animals, Nelson ('33), Mason ('33) and P'an, Van Dyke, Kaunitz and Slanetz ('49) reported an increase in gonadotropin, but Rowland and Singer ('36) and Drummond, Noble and Wright ('39) described that deficiency of vitamin E caused a decrease in luteinizing or ovulation producing substance but not in follicle stimulating substance. In our laboratory, Sakakura ('60) observed no influence in castrated mouse injected with vitamin E. Generally, it may be assumed that degeneration of testis by E-deficiency secondarily causes the castration changes in the anterior pituitary. Barrie ('37) thought that lack of vitamin E disturbed the formation of gonadotropic, thyrotropic and galactotropic hormones. Gierhake ('33) stated vitamin E controled the endocrine functions of the anterior pituitary. Further, Rowland and Singer ('36) and Beckmann ('55) presumed that pituitary might be the primary receptor of vitamin E because of containing large quantities of vitamin E in pituitary gland. While, there were some reports which vitamin E reinforced the action of testosterone (Adamstone, '41; De Watteville et al., '48) and follicle hormone (Schneider, '39). It was not distinct from this data whether vitamin E influenced primarily on pituitary or not.

Summary

Thirty-four immature and 32 mature mice of both sex were injected daily with water-soluble vitamin E ("E-230-001" prepared by Eisai Co., Ltd.). Immature group were injected with 10 mg from 23 days of age, and mature group with 20 mg about 90 days of age, for periods of 5, 10, 15 and 20 days.

1) Anterior pituitary: Acidophiles did not show the marked changes, but in some cases, large sized or small sized cells were somewhat more frequently observed in them than in the controls. Delta cells in immature group showed generally a slight decrease in cytoplasmic granulation, although, after 10 injections, heavily granulated delta cells were somewhat numerously found than in the controls. In initial stage of mature group, densely granulated delta cells were somewhat more numerous seen than in the controls, while, at late stage, they indicated a slight increase of gran-
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ules. Beta, theta and chromophobe cells did not differ in number, size and cytoplasmic granulation from those of the controls.

2) Testis: In immature group, meiotic division and maturing and mature sperms were generally more numerously observed than in the controls.

Interstitial cells indicated an increase in size and vacuolation. In mature group, germinal cells did not show any marked changes in general, although after 10 injections mature sperm indicated a slight increase in number, and after 15 injections, in some cases, the tubules containing sperms were slightly less found than in the controls. However, interstitial cells indicated an increase in size and vacuolation.

3) Ovary: Histological changes in ovary were generally not distinct. At initial stage of the experiment, slightly advanced corpora lutea which are not found in the controls were observed in some cases of immature group, and also in mature group, corpora lutea at advanced stage were somewhat more frequently seen than in the controls in initial stage of the experiment. Following 15 injections in immature group, involutions were somewhat less found than in the controls, but following 15 injections in mature group, they were somewhat more frequently found than in the controls.

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Literature Cited


Explanation of Figures

Plate I. Immature group.

Fig. 1. Male pituitary of a control mouse. Delta cells and acidophile cells are well-granulated. Zenker-formalin acetic acid fixation. Azan stain. ×1000.

Fig. 2. Male pituitary of a control mouse. Delta cells are well-granulated. Zenker-
formalin acetic acid fixation. PAS reaction. ×1000

Fig. 3. Male pituitary following 10 injections. Densely granulated delta cell is seen. Zenker-formalin acetic acid fixation. PAS reaction. ×1000

Fig. 4. Testis of a control animal. Zenker-formalin acetic acid fixation. Hematoxylin-eosin stain. ×240

Fig. 5. Testis following 10 injections. Mature spermatozoa are somewhat frequently seen. Zenker-formalin acetic acid fixation. Hematoxylin-eosin stain. ×240

Fig. 6. Ovary following 5 injections. Advanced corpus luteum is found. Zenker-formalin acetic acid fixation. Hematoxylin-eosin stain. ×60

Plate II. Mature group

Fig. 7. Male pituitary of a control mouse. Zenker-formalin acetic acid fixation. ×1000

Fig. 8. Male pituitary following 15 injections. Delta cells are degranulated and large in size. Zenker-formalin acetic acid fixation. PAS reaction. ×1000

Fig. 9. Testis of a control mouse. Zenker-formalin acetic acid fixation. Hematoxylin-eosin stain. ×320

Fig. 10. Testis following 10 injections. Mature sperms increase in number. Zenker-formalin acetic acid fixation. Hematoxylin-eosin stain. ×320

Fig. 11. Ovary of a control animal. Involutions are often seen in deep zone. Zenker-formalin acetic acid fixation. Hematoxylin-eosin stain. ×120

Fig. 12. Ovary following 15 injections. Involutions are frequently seen. Zenker-formalin acetic acid fixation. Hematoxylin-eosin stain. ×120
Plate 1

1. Yoshioka