Plasma Estradiol Concentrations and Effect of HCG on Plasma Estradiol and Testosterone in Normal Subjects and Patients with Endocrine Disorders

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Synopsis

Plasma estradiol concentrations were determined by radioimmunoassay in various endocrine disorders using antiserum to estradiol-17β succinyl bovine serum albumin. Clinical significance and diagnostic value of plasma estradiol were assessed in hypothalamic-pituitary, adrenal and gonadal disorders. In general, estradiol concentration was correlated well with the degree of sexual maturity and was of great diagnostic use. Plasma estradiol in females mainly originated from the ovary, while the testis is the principal source of estradiol in males. The adrenal gland seemed to play a minor role as a source of estradiol at least in normal males and females. The role of estradiol in gynecomastia and in liver disease was also investigated. More than a half of the cases with gynecomastia had elevated concentrations of plasma estradiol, which probably explains the pathogenesis of this manifestation. Cirrhotic patients showed frequently hyperestrogenemia probably due to delayed disappearance of estradiol. In the study of stimulation with human chorionic gonadotropin (HCG), 3,000 IU daily for three days in ten normal men, the peripheral concentrations of estradiol showed maximum and fourfold increases 24 hours after the 1st injection of HCG. The testosterone levels, on the other hand, increased stepwise and reached a maximum of about two times preinjection levels 24 hours after the 3rd injection. In gonadal disorders, HCG produced various patterns of plasma estradiol and testosterone in accordance with the gonadal conditions and dissociated response patterns of both sex hormones were frequently found. The determination of plasma estradiol was useful in the study of the function of not only the ovary, but also the testis and the simultaneous measurement of plasma estradiol and testosterone after HCG administration presented interesting informations about pathophysiology of gonadal disorders.

The clinical applications of plasma estradiol determination using radioimmunoassay method have been commenced for elucidating pathogenesis and for proper diagnosis of gonadal disorders (Jenner et al., 1972; Bidlingmaier et al., 1973; Ruder et al., 1974). At present, the knowledge on plasma estradiol levels in various endocrine disorders is still fragmentary and clinical significance of peripheral estradiol levels is not always easily interpreted. As an aid to facilitate the interpretation of basal levels of sex hormones, stimulating procedure of the gonads with HCG is a commonly adopted method. However, response pattern to HCG with plasma estradiol as an index is not fully established in both sexes and requires further investigations. Of these, stimulation study of estradiol with HCG in males seems especially interesting considering that testosterone is a main precursor of estradiol.

The present paper describes plasma es-
tradiol concentrations in various endocrine disorders for the evaluation of ovarian and testicular functions and also the response patterns of plasma estradiol and testosterone to HCG administration in normal men and male patients with gonadal disorder.

Materials and Methods

1) Subjects
Plasma of normal males (age 20 to 40 years) and normal females (age 20 to 35 years) with regular menstrual cycle was obtained from healthy volunteers. Patients with various endocrine disorders, as shown in Figure 2, were under care in the Nagasaki University Hospital.

Synthetic LH-RH, 100 μg, was given intravenously to 9 normal adult males. Blood samples were taken before and 30, 60, 90 and 120 minutes after the injection. LH and FSH levels in blood were determined by double antibody method of radioimmunoassay.

HCG, 3,000 IU, was given intramuscularly at 8 a.m. daily for 3 days to 10 normal adult men and 12 male subjects with gonadal disorder. Samples for plasma estradiol and testosterone were drawn before the injection and 6 and 24 hours after each injection of HCG.

2) Estradiol Determination
Plasma estradiol was measured by radioimmunoassay technique which utilized an antiserum produced by immunization of rabbit with estradiol-17β-hemisuccinate coupled with bovine serum albumin. Without any pretreatment, the antiserum was diluted to 1:10,000 in 0.1M phosphate buffer (pH 7.0) containing 0.1% gelatin.

Assay procedure. After the addition of 2,000 dpm of 3H-estradiol-17β (SA 46.6 Ci/mM, New England Nuclear Corporation) to monitor procedural loss, one to 3 ml of plasma was added and extracted twice with 15 ml of fresh ether. A microcolumn of Sephadex LH-20 (Pharmacia) was prepared for purification of the combined ether extract. The column was 7×100 mm and was packed to a height of 70 mm with Sephadex LH-20 in benzene-methanol solution (85:15 V/V). The flow rate was 200 μl/min. The estradiol fractions were collected into assay tubes and evaporated to dryness. A series (1.56 to 800 pg) of standard estradiol-17β (Sigma Chemical Company) in triplicate and estradiol fractions of samples were kept in an ice bath. One hundred μl of diluted antiserum was added to each of standard and sample tubes. The mixture was gently agitated and 100 μl of 3H-estradiol-17β solution (20,000 dpm/100 μl in 0.1% gelatin phosphate buffer) was added. The tubes were incubated at 4°C for 21 hours. Then, 1 ml of dextran-coated charcoal solution (125 mg charcoal and 25 mg dextran T-70/100 ml phosphate buffer) was added to the tubes. The tubes were centrifuged at 2,500 r.p.m. for 10 minutes at room temperature. Five hundred μl of supernatant was transferred into a counting vial containing 10 ml scintillation fluid. Fifty percent gradient was obtained for the range from 1.56 pg to 800 pg in standard curve.

Reliability of the method. The water blank values were below 1.56 pg. The values of estradiol in 3 ml of plasma obtained from four completely hypophysectomized patients ranged from undetectable to 3.1 pg per ml. Various amounts of pure estradiol ranging from 25 to 200 pg were added to 2 ml of male pooled plasma and processed by extraction, column chromatography and assay. As a result, linear relationship was obtained between the estradiol added and that recovered and quantified. The coefficient of variation from repeated analysis of pooled male plasma was 13.6%. As for sensitivity, it was found that the difference between 1.56 pg and zero of estradiol was significant (p<0.05).

3) Testosterone Determination
Plasma concentration of testosterone was determined by competitive proteinbinding method (Maeda et al., 1969)

Results

Normal subjects.
The results of the determination of plasma estradiol in normal subjects are shown in Figure 1. Normal values in 28 healthy males varied from 16.3 to 49.6 pg per ml, with a mean of 30.3±12.6 (SD) pg per ml. Normal values in 14 healthy females with regular menstrual cycle were 74.4±37.0 pg per ml in the follicular phase, 276.2±177.9 pg per ml in midcycle and 174.9±63.0 pg per ml in the luteal phase.

Plasma estradiol concentrations of endocrine disorders.
Plasma estradiol concentrations of various endocrine diseases are shown in Figure 2. The values obtained from subjects with hypophysectomy, panhypopituitarism, isolated gonadotropin deficiency, castration and
Fig. 1. Plasma estradiol concentrations in normal males and females.

Fig. 2. Plasma estradiol concentrations in endocrine diseases. Shaded areas represent mean ± SD in normal males and in normal females.
postmenopausal women were significantly low (p<0.005) except for a male partially hypophysectomized for pituitary tumor (28.9 pg/ml) and a female with unilateral oophorectomy (202.2 pg/ml in the luteal phase). The low values of estradiol were also seen in hypothalamic syndrome with hypogonadism, a male patient with anorexia nervosa, a male pseudohermaphroditism due to 17α-hydroxylase deficiency, Klinefelter’s syndrome, Noonan’s syndrome and Turner’s syndrome. In 2 cases of Laurence-Moon-Biedl syndrome and 3 cases of myotonic muscular dystrophy, those with hypogonadism showed low values of estradiol, while those without hypogonadism normal values. Two patients with idiopathic precocious puberty, 3-year-old boy and 7-year-old girl (follicular phase), had plasma estradiol of 36.9 and 43.4 pg per ml respectively. Each female case of isolated ACTH deficiency, adrenogenital syndrome due to 21-hydroxylase deficiency and total adrenalectomy due to bilateral pheochromocytoma, under treatment with glucocorticoid, had plasma estradiol of 457.7 (luteal phase), 62.6 (follicular phase) and 208.9 (luteal phase) pg per ml respectively. One of 2 patients with Addison’s disease had a high value of estradiol. Of the 5 patients with Cushing’s syndrome before operation, a man with adrenal hyperplasia and a male patient with adrenal adenoma showed low values and the estradiol concentrations of female patients with adrenal adenoma studied in the luteal phase were normal. In two cases with testicular feminization, one patient with complete type had normal male values of plasma estradiol (29.7 pg/ml) and testosterone (538.3 ng/100 ml) and the other patient with incomplete type plasma estradiol (23.5 pg/ml) and testosterone (763.0 ng/100 ml). In 20 male patients with liver cirrhosis, plasma estradiol level ranged from 21.0 to 103.9 pg per ml, with a mean of 57.4 pg per ml. The values in 14 patients were higher than those seen in normal males. In 8 subjects with gynecomastia of various etiologies, a male with hyperthyroidism showed 57.8 pg per ml, a hypertensive patient under treatment with spironolactone 57.4 pg per ml and a 73-year-old hypertensive patient under treatment with reserpine 10.8 pg per ml. Five patients due to unknown cause, ranged from 15 to 25 years of age, had plasma estradiol of 68.9, 63.7, 63.1, 41.1, and 29.8 pg per ml. Five of the 8 subjects had high values of estradiol.

Effect of LH-RH.

Figure 3 shows the effect of synthetic LH-RH on serum LH, FSH and plasma estradiol concentrations in nine normal men.

![Fig. 3. Effects of 100 μg of LH-RH on serum LH, FSH and plasma estradiol in 9 normal male subjects. Values are expressed as mean ±SD.](image-url)
One hundred µg of LH-RH produced approximately eight- and threefold increases above resting level in LH and FSH respectively, but an only slight increase in plasma estradiol.

Effect of HCG.

The effect of HCG on plasma estradiol and testosterone in 10 normal adult men and 12 male subjects with gonadal diseases is shown in Table 1 and 2. In normal males, as shown in Figure 4, the mean plasma estradiol increased to a maximum of 127.7±47.0 pg per ml (SD) 24 hours after HCG administration which was approximately 4 times as high as the original mean value, although No. 9 and 10 showed the highest levels 6 hours after the 2nd injection and No. 5 showed maximum response to HCG on the 3rd day. Until 6 hours after the 2nd administration of HCG, the mean values of plasma estradiol maintained high level and then decreased gradually. Testosterone concentrations, on the other hand, responded differently to additional HCG stimulation and increased stepwise until the 4th day. The testosterone concentrations reached a maximum of about 2 times preinjection level 24 hours after the 3rd injection of HCG.

In gonadal diseases, the responses to HCG varied with the degree of the gonadal functions. Four cases with isolated gonadotropin deficiency had low levels of estradiol and testosterone and responded slightly to the 2nd or 3rd administration of HCG. A 56-year-old patient, without any treatment for a long time, hardly responded to HCG. In Klinefelter's syndrome and myotonic muscular dystrophy, No. 6 and 8 showed normal increases of estradiol after HCG, though responses of testosterone were poor. A patient with testicular feminization showed normal increase of testosterone and reduced response of estradiol. Two subjects with orchiectomy failed to respond to HCG.

Discussion

The mean and range of peripheral plasma estradiol concentrations in normal adult males and females obtained by the present method were similar to those reported by other workers (Mikhail et al., 1970; Wu and Lundy, 1971; Abraham et al., 1972; Emment et al., 1972; Saez et al., 1972; Wright et al., 1973). Although it is extremely important to know the sources of plasma estradiol in males and females for the understanding of normal and pathological conditions, there exist several difficulties to be solved such as multiple precursors of estrogens, inter-
Table 1. Effect of HCG on plasma concentrations of estradiol and testosterone in normal men

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Mean±SD

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* E₂=Estradiol (pg/ml), ** T=Testosterone (ng/100 ml)

Table 2. Effect of HCG on plasma concentrations of estradiol and testosterone in Gonadal Disorders

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* E₂=Estradiol (pg/ml), ** T=Testosterone (ng/100 ml)
conversions among estrogens, abnormal biosynthetic pathways and so on. Along this line, plasma estradiol concentrations of subjects with castration, hypophysectomy, postmenopause and bilateral adrenalectomy are able to give to a certain extent simple and effective informations about knowing the sources of plasma estradiol.

In females, the plasma concentrations of estradiol observed in the subjects with bilateral oophorectomy and postmenopausal women in this series were extremely low. Furthermore, the far less contribution of the adrenal gland to plasma estradiol was confirmed by the normal concentration of estradiol seen in a bilaterally adrenalectomized female patient maintained on cortisone, and also by high normal estradiol in a female patient with isolated ACTH deficiency. Saez et al. (1972) showed that adrenal suppression with dexamethasone and stimulation with ACTH did not influence the concentration of plasma estradiol. These suggest that plasma concentration of estradiol is for the most part maintained by the ovary in females and the adrenal glands contribute to a much less degree. A normal level of plasma estradiol shown in a patient with unilateral oophorectomy indicates that one ovary is capable of maintaining normal concentration of plasma estradiol. From these results, it is concluded that plasma estradiol in normal females mainly originates from the ovary and is an excellent index of the ovarian function.

In males, the role of the human testis in estrogen production has been controversial for many years. Recently, the direct secretion of estradiol by the human testis has been shown by Kelch et al. (1972), Longcope et al. (1972), Saez et al. (1972) and Baird et al. (1973) on the basis of measurement of estradiol in spermatic venous blood. The castrated patients presented here revealed extremely low levels of plasma estradiol in the presence of both normal adrenal glands. Intramuscular administration of HCG in normal males, on the other hand, markedly increased the plasma estradiol level (Longcope, 1973; Doerr et al., 1974; Weinstein et al., 1974) and no low concentration of estradiol was found in two male patients with Addison's disease. These results suggest that the testis contributes to most of plasma estradiol concentration at least in normal males and estradiol as well as testosterone seems well to reflect testicular function.

A way of assessing the clinical significance of measuring plasma estradiol should be based on the correlation between estradiol concentration and the degree of sexual maturity. In this sense, the low concentrations of plasma estradiol observed in hypogonadism of various causes, hypopituitarism, hypothalamic lesions and castration in both sexes, were thought fairly to reflect the hypofunction of the testis or the ovary.

Two cases of precocious puberty were associated with an elevation of plasma estradiol in comparison with normal prepubertal subjects. One of them, a 3-year-old boy showed an adult level of estradiol and testosterone (435.0 ng/100 ml) and the testicular biopsy revealed well-developed seminiferous tubules and the Leydig cells comparable to those of adult males. Jenner et al. (1972) reported that in patients with precocious puberty, plasma estradiol levels correlated well with the stage of sexual development and clinical course and might be of great use in the determination of diagnosis and prognosis of sexual precocity. The physiological significance of low estradiol concentration in two males with Cushing's syndrome and high estradiol concentration in a male with Addison's disease is not clear and waits for further evaluation.

The pathogenesis of gynecomastia has long been debated and multiple factors have been suspected. Recently, the role of estrogen in hyperthyroid patients with gynecomastia was studied by measuring plasma estradiol. The result revealed that gyno-
comastia was possibly explained by elevated plasma estrogens (Chopra et al., 1972; Ber
covici and Mauvais-Jarvis, 1972). On the
other hand, in the study of pubertal gyneco-
comastia, no elevation of plasma estradiol
was reported (Bidlingmaier et al., 1973).
The result in the present paper showed that
plasma estradiol concentration in 5 of the
8 patients was higher ($p<0.005$) than
normal. In study of 20 patients with
liver cirrhosis, the relation between plasma
estradiol concentrations and presence of
gynecomastia was inconsistent. Judging
from these results, the etiology of gyneco-
comastia may be diverse and a part of gyneco-
comastia may be explainable by high con-
centration of estradiol.

The high frequency of testicular atrophy,
reduced libido, gynecomastia and spider an-
gioma in men with liver cirrhosis has long
been recognized. The elevation of circulat-
ing estrogens due to abnormal estrogen
metabolism in this disease have been impli-
cated in the pathogenesis of these symp-
toms. However, studies regarding the concen-
trations of plasma estradiol so far have shown inconsistent results (Kent et al.,
1973; Galvão-Teles et al., 1973; Chopra
et al., 1973). The present results demon-
strated that high level of plasma estradiol
was recognized in 14 of the 20 patients
with liver cirrhosis. The levels of es-
tradiol tended to correlate with the values
of serum bilirubin, indocyanine green and
bromsulphalein retention rate. Furthermore,
in study of intravenous infusion of 200 $\mu$g
of estradiol-17β in the patients, the disap-
pearance of exogenous estradiol was pro-
longed in comparison with that of normal
males (unpublished data). Therefore it is
likely that in patients with liver cirrhosis,
hyperestrogenemia frequently exists and that
delayed disappearance of estradiol may
result in an elevation of plasma estradiol.

In the stimulation study of the pituitary-
gonadal axis, the administration of LH-RH,
100 $\mu$g, was an inadequate procedure to
increase concentrations of plasma estradiol
and HCG produced an marked elevation of
estradiol. The response patterns of plasma
estradiol and testosterone after HCG admin-
istration to normal adult men are interesting
from two points of view; the one is a dis-
pairity between the times reaching maximum
level of estradiol and testosterone and the
other is a difference of increasing rate. No
study in detail about the response pattern
of plasma estradiol after HCG stimulation
was reported. Longcope (1973) described
that plasma estradiol was higher on 3rd
day than on 5th day in the study of
administration of HCG, 3,000 IU, for 4
days. The present data of serial determi-
nations showed that plasma estradiol level
reached maximum 24 hours after the ad-
ministration of HCG and the additional
stimulation on the 2nd and 3rd day did
not give any further increase of plasma es-
tradiol. An early appearance of maximum
estradiol level might mean that the aro-
matizing process responsible for testosterone
conversion to estradiol was rapidly acceler-
ated by HCG in the presence of abundant
estradiol precursor. It was reported that
HCG stimulated the aromatization process
in human placenta (Cedard et al., 1970).
Moreover, the capacity of activated aro-
matizing enzyme possibly reached to the
limit on the first day with no further in-
crease on the subsequent days, and this
limitation might result in quite different
response patterns between estradiol and tes-
tosterone to the stimulation with HCG. It
is uncertain whether the limit of aromatiza-
tion process may well explain the significant
lower level ($p<0.005$) of the 4th day es-
tradiol.

Among the pathological conditions stud-
iied, the cases of gonadotropin deficiency
showed slow and late rising of plasma es-
tradiol and testosterone after HCG and no
early appearance of maximum levels of es-
tradiol as seen in normal men. The small
and late response of plasma estradiol pro-
bably reflects extremely low resting level of testosterone and its small increase after HCG stimulation. In addition, it is interesting to assume that a prolonged deficiency of pituitary gonadotropin is responsible for decreased aromatizing activity resulting in the poor response of plasma estradiol. Cases of hypergonadotropic hypogonadism, on the other hand, showed the different patterns from those of gonadotropin deficiency. In cases of Klinefelter's syndrome (Ruder et al., 1974) and myotonic muscular dystrophy, some patients showed normal response of estradiol to HCG, though the increase of testosterone was relatively small. This finding might be due to both relatively abundant testosterone which is enough for the estradiol production compared to that of gonadotropin deficiency and an increased conversion of testosterone to estrogen. Gabrilove et al. (1970) suggested that there was an increased conversion of androgens to estrogens in Klinefelter's syndrome and it might be facilitated both by the overproduction of pituitary gonadotropin and by the abnormal genetic sex constitution. A case of testicular feminization conversely showed normal increase of testosterone and somewhat reduced response of estradiol. Kelch et al. (1972) suggested that markedly elevated secretion of estradiol from the testis and decreased peripheral conversion resulted in normal concentration of peripheral estradiol in testicular feminization. It seems that the present result coincides with theirs.

Among gonadal disorders, there were frequently dissociated response patterns of testosterone and estrogen after HCG administration. Therefore the assessment of the Leydig cell activity requires measurement of both sex hormones and not only the resting levels of these hormones, but also the response patterns after HCG provide interesting informations about gonadal disorders.

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