PLASMA OESTROGENS AFTER THE MENOPAUSE

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A Thesis submitted to the University of London for the degree of Doctor of Philosophy, 1978.

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ABSTRACT

The aims of this study were to determine accurately the oestrogen status of the postmenopausal woman, the sources and mechanisms regulating oestrogen production, the relation of plasma oestrogens to climacteric symptoms and the effects of administered oestrogens on plasma oestrogen concentrations by employing intensive hormone profiling techniques.

The results showed that the major circulating deficiency resulting from primary ovarian failure was of oestradiol and that oestrone was, quantitatively, the dominant unconjugated oestrogen circulating in postmenopausal women. Plasma androgen and oestrogen concentrations in ovariectomised and postmenopausal women before and after dexamethasone treatment for one week, suggested that the postmenopausal ovary was an insignificant source of plasma steroids when compared with the adrenal contribution. Studies of women infused tetracosactrin or androstenedione after dexamethasone treatment for one week, showed that plasma oestrogens after the menopause could be largely produced by peripheral aromatisation of adrenal androstenedione. As there was a positive correlation of the fat mass and the plasma oestrone and oestradiol concentrations, adipose tissue may be the major site of extraglandular aromatisation. Other studies showed that production in, or the release from, the aromatising tissues was prolonged and possibly episodic, especially as plasma oestrone and oestradiol concentrations fluctuated asynchronously throughout the day.

Postmenopausal women with superficial dyspareunia had significantly lower mean plasma oestradiol, but not oestrone, concentrations when compared with the levels in asymptomatic women. There was also a highly significant correlation of the karyopyknotic index and the plasma oestradiol, but not oestrone, concentration in another group of women. Thus, oestradiol is, biologically, the most important plasma oestrogen in women after the menopause. The presence or timing of flushes was not, however, related to actual levels or fluctuations in plasma hormone concentrations. Finally this study showed that orally administered oestradiol valerate was absorbed and metabolised like piperazine oestrone sulphate, and not like oestradiol administered parenterally.

PART 1

Introduction, objectives, and critique and description of the methodology.

Summary: In this part, the current knowledge of the production and metabolism of plasma oestrogens after the menopause is reviewed. Then the objectives of this study are described: the accurate determination of the oestrogen status of postmenopausal women, the sources and mechanisms regulating oestrogen production, the relation of plasma oestrogens to symptoms usually attributed to ovarian failure and the effects of oestrogen therapy on oestrogen status. Finally, a critique of the methodology, and particularly the intensive hormone profiling technique employed in this study precedes a detailed description of the clinical, laboratory and statistical methods.

A) INTRODUCTION

The menopause is defined in the Oxford English Dictionary as the final cessation of menstruation. The menopause occurs during the climacteric which is the phase in the aging process of women which marks the transition from the reproductive stage of life to the non-reproductive stage (Utian and Serr, 1976). These and other relevant terms applicable to this study are summarised in Figure 1.1.

The menopause is a consequence of primary ovarian failure, which is caused by depletion of primordial follicles from the ovary. Each primordial follicle consists of a single layer of granulosa or pre-granulosa cells (which are specially differentiated ovarian stromal cells) surrounding an oocyte. The oocytes of the primordial follicles, which are formed only during intrauterine life, are primordial germ cells (oogonia) which have entered the prophase of the first meiotic division. The number of oocytes in the ovary declines exponentially from the fifth month of intrauterine life (Figure 1.2) and shortly after birth, oocytes which have not reached the diplotene ('resting') phase of meiosis, along with remaining oogonia, are eliminated by the process of atresia. Although in each ovulatory cycle, about 20 primordial follicles are stimulated to become Graafian follicles and degenerate shortly after one of them ovulates, the decline in the number of primordial follicles during the reproductive phase of life is due mainly (99.9%) to the process of atresia (Baker, 1971).

Some atresia of oocytes occurs in all species, but complete elimination of primordial follicles occurs only in humans (usually by the age of 60 years (Hloch, 1961)), and in inbred strains of mice (Mintz, 1959). The nature, cause and timing of this atresia

Figure 1.1. Definition of terms of stages of life about the menopause (from Jaszmann, 1976).

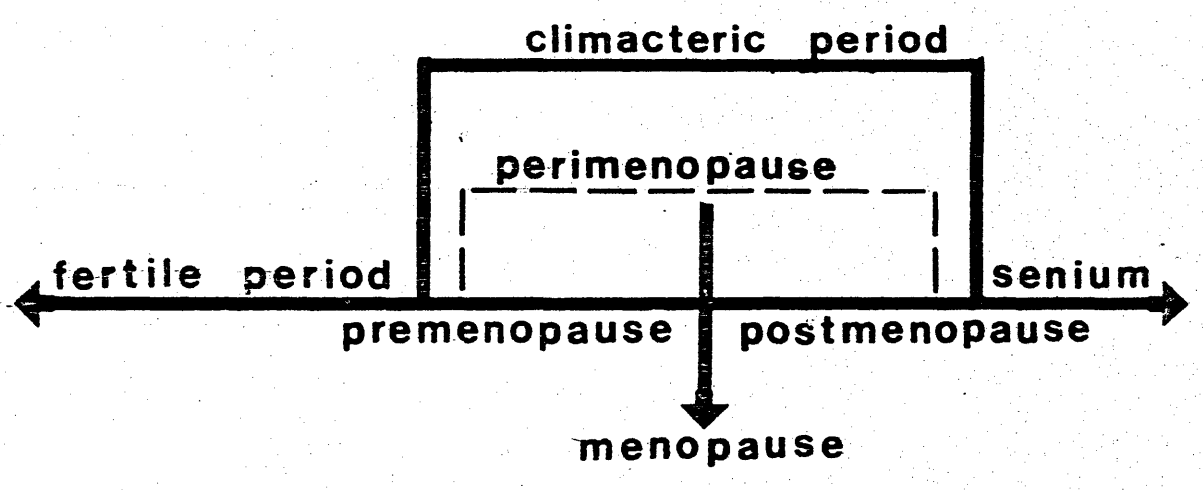
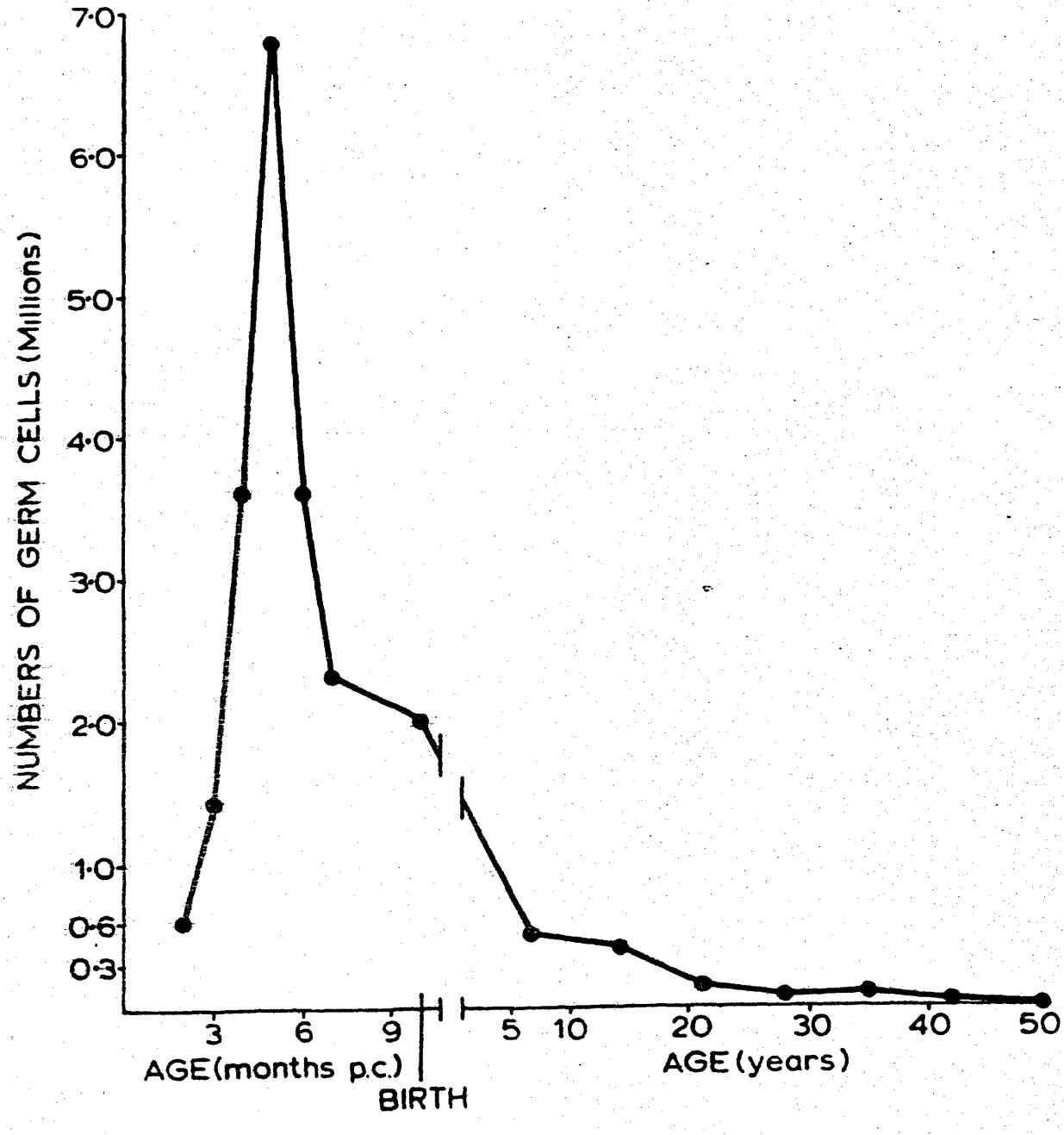

climacteric period
perimenopause
fertile period
senium
premenopause
postmenopause
menopause

Figure 1.2. Fluctuations in the total population of germ cells in the human ovary during reproductive life (from Baker, 1971).


NUMBERS OF GERM CELLS (Millions)
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0·6
0·3
3
6
9
5
10
20
30
40
50
AGE (months p.c.)
BIRTH
AGE (years)

are not understood although genetic factors are probably important. The loss of an X-chromosome such as in Turner's syndrome is associated with premature ovarian failure, usually around the time of birth (Singh and Carr, 1966) and X-chromosome loss in ovarian tissue increases with age (Fang et al., 1975). Other factors known to accelerate the rate of atresia include autoimmune disease (Golonka and Goodman, 1968), irradiation (Nathanson et al.,1940), cytotoxic drugs (Rose and Davis, 1977) and viral diseases (Morrison et al., 1975).

The elimination of oocytes results in postmenopausal women being sterile. However, the depletion also of the surrounding follicles has important endocrine consequences because during the reproductive phase of life, the Graafian follicles and corpora lutea which develop from them, secrete oestrogens.

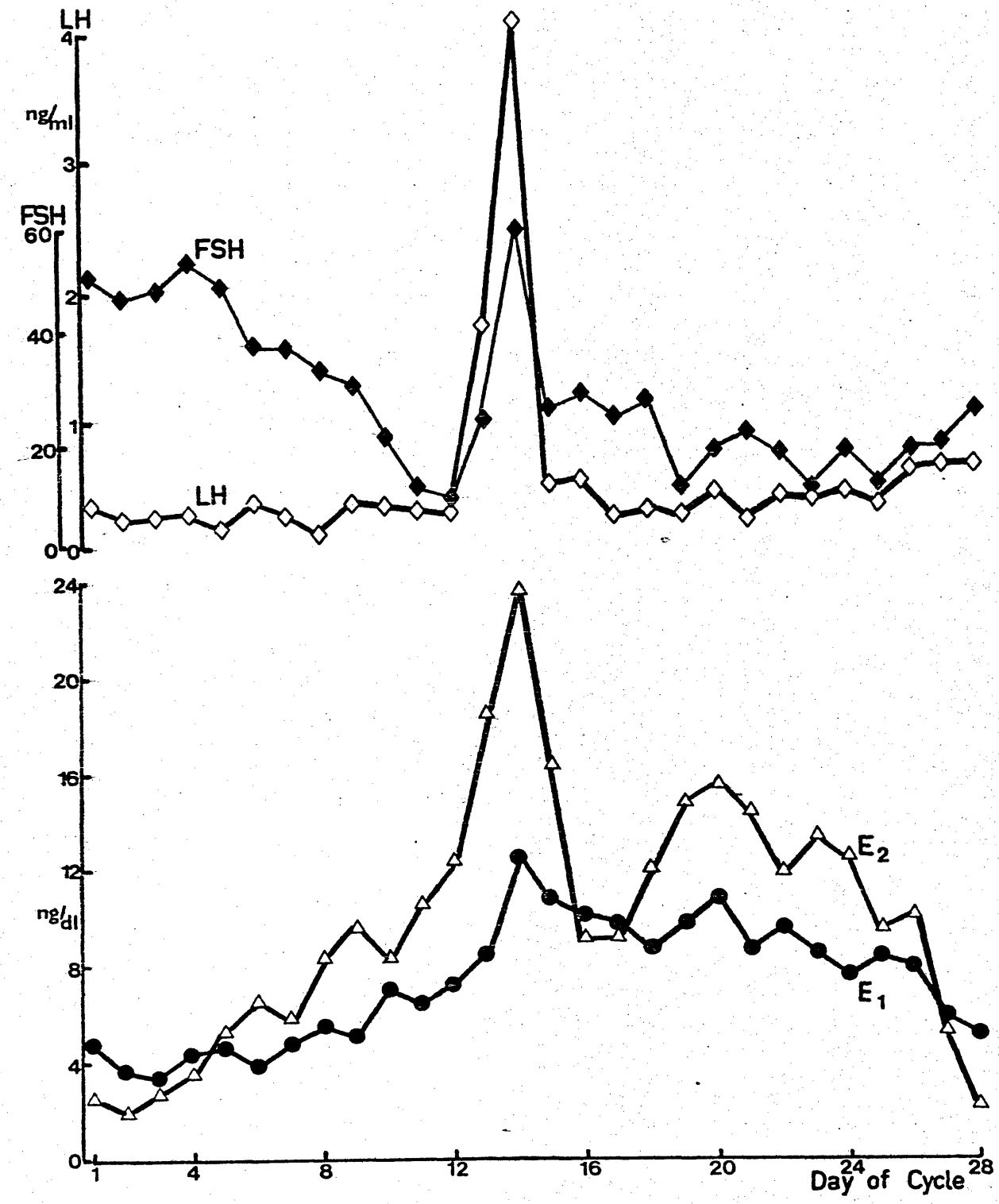
Oestrogens are steroid hormones with 18 carbon atoms and in the female they are primarily concerned with reproduction, especially the growth and function of the reproductive tract and the secondary sex characteristics. The cytoplasm of the cells of target tissues (i.e. those derived from the Müllerian duct and urogenital sinus, and of the breast, adenohypophysis and hypothalamus) contain highly specific proteins (receptors) which bind oestradiol-17β with much higher affinity than oestrone. Intracellular oestradiol is derived primarily from plasma oestradiol, but a proportion may be produced by the intracellular reduction of oestrone which, in most biological systems, is a much weaker oestrogen than oestradiol (Fotherby, 1976). Transposition of the oestradiol receptor complex into the nucleus stimulates a specific cellular response which, for the epithelial cells of the urogenital tract and breast, is primarily proliferative. The metabolism of other tissues such as those in bone, skin and other

parts of the nervous system may also be affected by plasma oestrogens although the mechanism of action of oestrogens in these tissues is not understood.

The cyclopentenoperhydrophenanthrene ring nucleus of oestrogens is synthesised from acetate only in the stromal cells derived in the embryo from the median crest of the dorsal abdominal wall, i.e. in the adrenal cortex and the gonads. During the reproductive phase of life, 95% of circulating oestradiol is secreted by the Graafian follicles or the corpora lutea (Baird and Fraser, 1974) and primarily by the stromal layer which develops around the pre-granulosa layer (Gower and Fotherby, 1975). Although the cells of the Graafian follicles and corpora lutea also secrete oestrone, more than 50% of plasma oestrone in premenopausal women is derived from adrenal sources or the peripheral conversion of plasma oestradiol (Baird and Fraser, 1974). The plasma concentrations of oestradiol and of oestrone determined in daily blood samples throughout the ovulatory cycle, fluctuate in a characteristic fashion similar to that shown in Figure 1.3 .

The most characteristic effect induced by the increase in plasma oestrogen levels that results from follicular development is proliferation of the endometrium, from which menstruation ensues after the oestrogen levels later fall because of luteolysis. By the age of about 50 years, the population of primordial follicles has become severely depleted, development of Graafian follicles ceases and thus, cyclical proliferation of the endometrium can no longer occur: women are then postmenopausal. The age of menarche (Wood, 1971), smoking (Jick et al., 1977), parity (Soberon et al., 1966) and marital, socioeconomic, racial and hereditary factors (Jąszmann et al., 1969; McKinlay and Jeffries, 1974; Frere, 1971; Damon et al., 1969)

Figure 1.3. Plasma oestrone (E_1), oestradiol (E_2), follicle stimulating hormone (FSH) and luteinising hormone (LH) in daily blood samples obtained from a normal premenopausal woman during a menstrual cycle (adapted from Hawkins and Oakey (1974) and Jacobs and Murray (1976)).


LH
4
ng/ml
3
FSH
60
FSH
2
40
1
20
LH
0
0
24
20
16
12
ng/dl
8
4
0
E2
E1
1
4
8
12
16
20
24
28
Day of Cycle

may affect the age of the menopause slightly. The age of the menopause was apparently also about 50 years in Greek and Roman times (Amundsen and Diers, 1970). Since then, of course the life expectancy of women has dramatically increased, especially in this century, and women in Western Europe and North America can now expect to live, on average, until about the age of 75 years (Jaszmann, 1976).

The secretion of oestrogens by the human Graafian follicle was first recognised using bioassay techniques based on the effects of oestrogen on the vaginal cytology of rodents (Allen and Doisy, 1923). The recent development of chemical techniques for studying hormones has enriched our knowledge of oestrogen metabolism in premenopausal women, and particularly plasma oestrogens and the factors regulating their follicular secretion: this has led to improved management of premenopausal women, particularly those with disorders of oestrogen production. Women are now, however, postmenopausal for almost as long as they are premenopausal yet our present knowledge of oestrogen metabolism in postmenopausal women is sparse in comparison with that in premenopausal women.

B) OBJECTIVES

The general purposes of this study were to determine

i) the concentrations of plasma oestrogens after the menopause and the factors which regulate their production from adrenal, ovarian and extraglandular sources after ovarian failure.

ii) the relation of plasma oestrogens to clinical effects of primary ovarian failure, especially as many symptoms have sometimes been attributed to the consequent oestrogen deficiency (Oram and Chakravarti, 1975; Campbell and Whitehead, 1976).

iii) the effects of postmenopausal oestrogen therapy on plasma oestrogen concentrations particularly because current regimes of 'hormone replacement therapy' are thought to cause endometrial carcinoma in some women (Ziel and Finkle, 1975; Smith et al., 1975; McDonald et al., 1977; Gray et al., 1977).

The ultimate motive of this study was to develop a firmer biochemical basis for the practical management of women after the menopause.

i) PLASMA OESTROGEN CONCENTRATIONS AFTER THE MENOPAUSE

The objectives of this part of the study were to investigate

a) the exact hormonal status of the postmenopausal woman.

b) the contribution of the postmenopausal ovary to plasma oestrogens.

c) the contribution of the adrenal cortex to plasma steroids.

d) the extraglandular production of plasma oestrogens from androgens.

a) THE HORMONAL STATUS OF THE POSTMENOPAUSAL WOMAN

The aim of this part of the study was to determine accurately the oestrogen, androgen and gonadotrophin status of women after the menopause. In the first reported study of plasma oestrogen concentrations in postmenopausal women, Frank et al.(1934) found that the 'oestrogenic substance' was undetectable by the Allen and Doisy test. However Fluhmann (1936) subsequently showed that 'oestrogenic substance' was detectable' in plasma, and furthermore, that the levels fluctuated from day to day. Bioassay techniques for determining plasma oestrogen concentrations were eventually superseded by more specific and sensitive chemical methods involving fluorimetry (Ichii et al., 1963) gas chromatography (Attal et al., 1967; Wotiz et al., 1967) and colorimetry (Brown et al., 1968), but these were not applied directly to the study of plasma oestrogens after the menopause.

In 1968, Baird developed a sensitive double-isotope derivative technique for determining plasma oestrone and oestradiol levels. Baird and Guevera (1969) then showed that, unlike premenopausal women, quantitatively the dominant unconjugated oestrogen circulating in postmenopausal women was oestrone. This important observation has since been confirmed by many other workers, who have usually employed saturation analysis techniques. However, the mean plasma oestrone concentration in the various studies has ranged from 2.2 $\pm$ 1.4(SD) ng/dl (n=13) reported by Nagai and Longcope (1971) to 7.1 $\pm$ 2.7 ng/dl (n=6) by Baird and Guevera (1969). A wide range of mean plasma oestradiol concentrations in women after the menopause has also been reported: from 0.45 $\pm$ 0.36 ng/dl (n=13) reported by Nagai and Longcope (1971) to 3.7 $\pm$ 4.3 ng/dl (n=11) by Campbell et al.(1976). As the standard

deviation of the means is often large, there must be a large variation in the levels for postmenopausal women. In many studies the mean plasma oestrone or oestradiol concentrations have been determined in random blood samples obtained from only a few women whose menopause usually occurred a variable number of years previously and/or whose weight may have been variable. The design of this study attempts to obviate possible variables which might influence the mean oestrogen concentration.

One reason for measuring plasma androstenedione and testosterone levels in this study was to determine if a deficiency of these androgens resulted from primary ovarian failure, especially because peripheral conversion of androstenedione may account for all plasma oestrone after the menopause (Grodin et al., 1973), and also because testosterone is sometimes prescribed to postmenopausal women for 'menopausal symptoms' attributed to ovarian failure (Studd et al., 1976). Abraham (1974) has calculated that the mean contribution of the premenopausal ovary to the peripheral levels of androstenedione and testosterone were 30% and 50% respectively. Ovarian venous effluent studies in premenopausal women undertaken by Horton et al. (1966), Gandy and Peterson (1968), Kirschner and Jacobs (1971), Lloyd et al. (1971) and Weisz et al. (1973) have demonstrated a significant gradient for these androgens across the ovary and similar gradients also occur after the menopause (Judd et al., 1974). A mid-cycle surge in the levels of androstenedione and testosterone has been observed by Lobotsky et al.(1964), Lloyd et al. (1971), Abraham and Chakmakjian (1973), Judd and Yen (1973), Abraham (1974) and Barberia and Thorneycroft (1974). However the evidence that the developing follicle is a

significant source of these plasma androgens in premenopausal women is conflicting because Dupon et al. (1973) and Valette et al. (1975) were unable to detect any alterations in the levels during the ovulatory cycle. Furthermore, Gandy and Peterson (1968), Grodin et al. (1973) and Greenblatt et al. (1976) have found that the peripheral levels of androstenedione and testosterone in postmenopausal women were not significantly different from levels in premenopausal women, although a difference has been observed by Vermeulen (1976) and Chakravarti et al. (1976).

Using a modified Ascheim-Zondek test, Fluhmann and Murphy (1939) first found that an anterior pituitary hormone substance circulated in the blood of postmenopausal women, the levels being undetectable in premenopausal women except during pregnancy. The delicate interrelationships that exist between pituitary gonadotrophin production and oestrogen production by the ovarian follicle have however, only been appreciated as a result of the development of sensitive biochemical techniques, particularly radioimmunoassay, for determining plasma FSH and LH concentrations. Compared with levels in premenopausal women, levels of FSH and LH in postmenopausal women are increased as a result of primary ovarian failure (Odell et al., 1967; Schalch et al., 1968; Jacobs and Murray, 1976). Although the increase is probably a consequence of the loss of follicular oestrogens, the increase in FSH, which may occur a few years before the menopause (Sherman et al., 1976), may alternatively result from decreased ovarian secretion of an inhibin-like substance, or a change in the hypothalamic pituitary sensitivity to the feedback effects of oestrogen (van Look et al., 1976). Recently Yen et al. (1972a, 1972b) and Root et al. (1972) have demonstrated that in

postmenopausal women the secretion of gonadotrophins is, as occurs in premenopausal women, episodic. One aim of this study was to determine the exact relationship between the production of gonadotrophins and of oestrogens after the menopause.

b) CONTRIBUTION OF THE POSTMENOPAUSAL OVARY TO PLASMA STEROIDS

The objective of this study was to examine the role of the postmenopausal ovary in the production of plasma oestrogens and their androgenic prehormones after the menopause. Presently, many gynaecologists regard the postmenopausal ovary as functionless, largely because no difference between postmenopausal and castrate women in their rate of excretion of oestrogens has been shown (McBride, 1957; Bulbrook et al.,1957, 1958; Brown et al., 1959; Gallagher et al., 1966; Poliak et al., 1971; Procope and Adlercreutz, 1973; Rome et al., 1973, 1977 and Grattorola et al., 1974). However Savard et al. (1965), Rice and Savard (1966), Plotz et al. (1967), Mattingly and Huang (1969), Berman et al. (1973) and Wortman et al. (1975) have shown that ovarian tissue obtained from postmenopausal women can synthesise androgens, particularly androstenedione and testosterone, from acetate, but can only synthesise oestrogens in limited amounts. A significant contribution to plasma androgen but not oestrogen concentrations has also been suggested from the results of ovarian gradient studies (Judd et al., 1974), and of dexamethasone suppression studies (Vermeulen, 1976). Grodin et al. (1973) have calculated that one third of plasma androstenedione in postmenopausal women is derived from the ovary, the remaining two-thirds being secreted by the adrenal cortex. However, if androgens formed in the ovarian stroma contribute significantly to oestrogen

production by their aromatization to oestrogens in extragonadal sites, as suggested by MacDonald et al. (1967), then gynaecologists might need to reconsider their policy of performing prophylactic ovariectomy at the time of hysterectomy for non-malignant disease in postmenopausal women, especially as the risk of developing ovarian cancer at this age is less than 1% (Cooke, 1976).

c) CONTRIBUTION OF THE ADRENAL CORTEX TO PLASMA STEROIDS

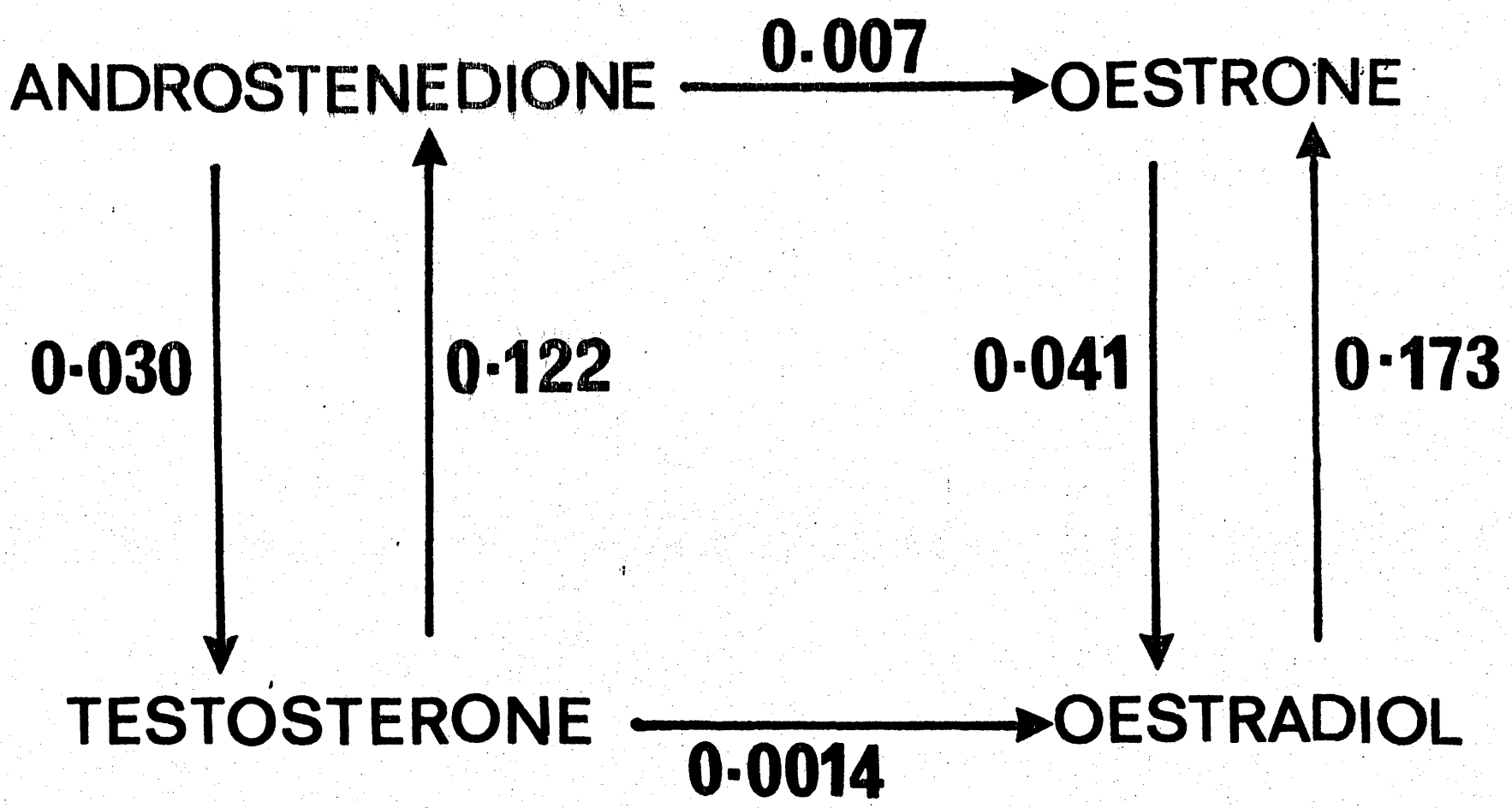
Fluhmann (1936) found similar amounts of oestrogens in the blood of castrate and postmenopausal women, and Parkes (1937) subsequently suggested that the adrenal cortex was the major source of oestrogen in postmenopausal women. In adrenalectomised, ovariectomised women, oestrogen excretion is unmeasurable (West at al., 1956). The administration of adrenocorticotrophin (ACTH) strikingly increases the excretion of oestrogens in ovariectomised women (Strong et al., 1956; West et al., 1958; Sandberg et al., 1957; Brown et al., 1959 and Barlow et al., 1969). However, in in vitro studies, tissue from the adrenal cortex has been shown to synthesise androgens but not oestrogens from acetate (Gower and Fotherby, 1975). Furthermore in a study of the concentrations of androgens and oestrogens in the adrenal venous effluent of a postmenopausal woman Baird et al., (1969) showed that the adrenal secretion was predominantly of androstenedione, and direct secretion of testosterone, oestrone and oestradiol was a relatively insignificant source of these plasma steroids. The aim of this study was to investigate the precise role of the adrenal cortex, and particularly its major trophic stimulus, ACTH, in the production of plasma oestrogens after the menopause.

d) EXTRAGLANDULAR PRODUCTION OF PLASMA OESTROGENS

Extraglandular production of oestrogens from androgens was first demonstrated by West et al. (1956) who detected oestrone and oestradiol-17β in the urine of two ovariectomised, adrenalectomised women after the administration of testosterone proprionate. MacDonald et al. (1967) showed from their isotopic infusion studies that the aromatisation of androstenedione at extraglandular sites may be sufficient to account for all urinary oestrone production after the menopause. Grodin et al. (1973) subsequently showed that plasma oestrone was derived only by the peripheral aromatisation of androstenedione. Figure 1.4 shows the peripheral interconversions and aromatisations of plasma androgens and oestrogens as determined by Longcope et al. (1969): The transfer constants are the fractions of precursor converted to product in premenopausal women. In addition, Olivo et al. (1973) have detected significant aromatisation of testosterone to oestrone.

Various tissues in the body have the enzymatic capacity to interconvert these sex-steroids (Ryan and Engel, 1953; Siiteri et al., 1972; Schweikert and Wilson, 1974). Although aromatisation has been demonstrated in brain (Naftolin et al., 1971), hair (Schweikert et al., 1975), fibroblasts (Schweikert et al., 1976), muscle (Bates, 1978, fat (Schindler et al., 1972; Bolt and Gobel, 1972) and liver (Smuk and Schwers, 1977), it is generally agreed that the peripheral aromatisation of androgens occurs predominantly in adipose tissue because of its relatively large mass (Nimrod and Ryan, 1975). The proportion of infused labelled androstenedione aromatised to oestrone correlates, in urinary conversion studies, with body weight (Siiteri and MacDonald, 1973; Hemsell et al., 1974; Rizkallah et al., 1975) and increased aromatisation occurs in women with endometrial

Figure 1.4. Peripheral interconversions of plasma androgens and oestrogens. The values shown are the blood transfer constants ($[\rho]_{BB}$) determined in premenopausal females by Longcope et al. (1968, 1969).


ANDROSTENEDIONE
0·007
OESTRONE
0·030
0·122
0·041
0·173
TESTOSTERONE
0·0014
OESTRADIOL

carcinoma (Siiteri and MacDonald, 1973; Hauskneckt and Gausberg, 1973) and in patients with cirrhosis (Gordon et al., 1975) and hyperthyroidism (Southren et al., 1974). The aim of this aspect of the study was to further examine the extraglandular production of plasma oestrogens after the menopause, and particularly, to investigate possible factors regulating the aromatisation of plasma androstenedione and testosterone to oestrone and oestradiol.

ii) TO DETERMINE THE RELATIONSHIP OF PLASMA OESTROGENS AND CLINICAL EFFECTS ATTRIBUTED TO OESTROGEN DEFICIENCY.

In the 19th century, the ovaries of young women were thought to influence other organs, and Tilt (1857, 1870) attributed 25 symptoms to the disappearance of the 'ovarian aura' at the menopause: some of these symptoms such as piles, pseudo-narcotism and chloro-anaemia are now known not to be due to direct effects of ovarian failure.

It is now generally recognised that primary ovarian failure is one direct effect of aging: the direct effects of ovarian failure must therefore be differentiated from the other direct effects of aging, as well as coincidental disease and environmental influences. The direct effects of primary ovarian failure are, as described previously, sterility and reduced oestrogen production.

Symptoms and signs may be attributed on clinical grounds to oestrogen deficiency after the menopause if they also often occur after ovariectomy and if they almost invariably respond to oestrogen treatment. The aim of this study was to relate plasma oestrogen concentrations to effects, especially clinical effects, attributed to oestrogen deficiency resulting from ovarian failure so that a rational approach to the prescription of postmenopausal oestrogen therapy might be developed. Presently, oestrogen deficiency that results from ovarian failure is definitely known, on the clinical grounds described above, to affect the urogenital tract, the vasomotor system and bone.

1) Urogenital Tract Effects: The classic consequence of the fall in oestrogen production that occurs after ovarian failure is atrophy of the genital tract epithelium. Amenorrhoea results from

endometrial atrophy , which only rarely causes any other symptoms or signs. Atrophy of the vaginal epithelium may cause superficial dyspareunia (defined as superficial dryness and/or discomfort during intercourse) in about 20% of postmenopausal women (Utian, 1972) and, in a few women, postmenopausal bleeding or discharge, due to atrophic vaginitis (Jeffcoate, 1975). Utian (1972) has shown that superficial dyspareunia is one of only two symptoms definitely attributable during the climacteric to oestrogen deficiency, according to the clinical criteria previously described. One aim of this study was to determine if there was a relationship between superficial dyspareunia and plasma oestrogen concentrations. The relation of superficial dyspareunia to postmenopausal oestrogen status determined either biochemically or cytohormonally has not been previously investigated.

After the menopause, atrophy of the lower urinary tract also occurs, and particularly of the distal urethra, which is derived, like the vagina, from the urogenital sinus (Zuckerman, 1940). However, only a few postmenopausal women develop urgency, urge incontinence or frequency which can be attributed on clinical grounds to oestrogen deficiency (Smith, 1976; Campbell et al., 1976).

Cytohormonal tests of oestrogen status such as the karyopyknotic index, maturation index and urinary sediment smear depend, like the original Allen-Doisy test, on an oestrogen-induced proliferation of atrophic lower urogenital tract epithelium. De Waard and Thijssen (1970) and Procope and Adlercreutz (1973) have found a positive correlation of the urinary excretion of oestrogens after the menopause and the oestrogen effect on the vaginal smear. However

Dove et al. (1971) and Stone et al. (1975) were unable to find a correlation of maturation indices and urinary or total plasma oestrogens respectively in postmenopausal women. Indeed many clinicians therefore consider that cytohormonal determinations are of no value in the management of postmenopausal women (Hammond, 1977). A further aim of this study was to determine the relation between plasma oestrogen concentrations and the cytohormonal status of postmenopausal women.

2) Vasomotor Effects: Flushes sweats and perspirations were once considered to be separate diseases of the 'change of life' (Tilt, 1870) but Maranon (1929) subsequently attributed all these episodic symptoms to vasomotor instability. Not all postmenopausal women develop these vasomotor symptoms, which may be distressing (Thompson et al., 1973). The exact nature and cause of flushes and/or sweats are presently obscure but most workers, with the notable exception of Mulley and Mitchell (1976), believe that altered oestrogen production during the climacteric is somehow involved in their pathogenesis. It is uncommon for these symptoms to persist for more than 10 years after the menopause (Thompson et al., 1973) and unlike superficial dyspareunia they are therefore regarded as a symptom only of the climacteric, and not of the senium. However, urine or plasma oestrogen levels, or cytohormonal patterns do not correlate with the occurrence of vasomotor symptoms after the menopause (Young et al., 1957; Dove et al., 1971; Stone et al., 1975; Jones et al., 1976; Chapman et al., 1976; Campbell et al., 1976; Abe et al., 1977). The aim of this study was to determine if there was any relationship between the occurrence of vasomotor symptoms and plasma oestrogens after the menopause.

3) Effects on Bone: Albright et al.(1940) first postulated that osteoporosis in old age could result from the earlier loss of the follicular source of oestrogens in ovarian failure. Although all postmenopausal women are, compared with premenopausal women, deficient in oestrogen, only some women develop symptomatic or radiological osteoporosis: these women have an accelerated loss of bone compared with the loss by normal women (Marshall and Nordin, 1977), more atrophy of the skin (McKonkey et al., 1963), axillary hair follicles (Smith, 1967) and lower urogenital tract (Gallagher et al., 1973). Although Riggs et al. (1973) did not detect a difference in plasma oestrogen levels, Marshall et al. (1977) found lower mean plasma oestrone (and androstenedione) levels in osteoporotic postmenopausal or ovariectomised women than in appropriately matched controls. Further definition of the exact relation between postmenopausal osteoporosis, which has a considerable morbidity and mortality (Gordan, 1977), and plasma oestrogen levels is required, especially because the prophylactic role of postmenopausal oestrogen therapy, and particularly the dose which just prevents osteoporosis has yet to be determined. However, the relation of plasma oestrogens and the effects of ovarian failure on bone has not been explored in this investigation.

4) Other Effects Attributed to Postmenopausal Oestrogen Deficiency: Many other effects have been attributed to the loss of the follicular source of plasma oestrogens after the menopause (Kupperman et al., 1953; Campbell and Whitehead, 1976; Oram and Chakravarti, 1975; Studd et al., 1976). However, on the clinical grounds previously described, Utian (1972a, 1972b, 1975)

found that only superficial dyspareunia and flushes could be attributed to oestrogen deficiency during the climacteric; all other symptoms such as loss of libido, mood changes, irritability and depression were not due to oestrogen deficiency. Campbell et al. (1976) also found in a study of climacteric postmenopausal women with various symptoms that oestrogen deficiency was significantly ($p < 0.01$) more effective than placebo in relieving only vaginal dryness and flushes: however, insomnia could also be relieved by oestrogen therapy if it was associated with flushes or sweats.

Skin-fold thickness can be correlated with the cytohormonal status of postmenopausal women (Nordin, 1978). Further studies in postmenopausal women are however necessary to distinguish the effects of oestrogen deficiency from the effects of age, other diseases and environmental influences before oestrogen therapy should be advocated for the relief of complications other than those described above.

iii) TO DETERMINE THE EFFECTS OF POSTMENOPAUSAL OESTROGEN THERAPY ON PLASMA OESTROGENS.

In 1889, Brown-Séquard described some remarkable effects of the self-administration of testicular extracts on intellect, and muscular, excretory and defaecatory powers, and he postulated that injections of ovarian extracts in old women 'would act like testicular extracts in old men'. In 1893 he stated 'of the ovaric liquid, I will only say that it acts with less power than the orchitic liquid.. However, 60 old women in Paris have derived benefit from its action according to an American lady physician, Mrs Brown. It would be important to make use of that special liquid in cases of removal of both ovaries, or of disease having destroyed them'. The prescription of oestrogen, the principle ingredient in 'ovaric liquid', is now common treatment for many women after the menopause or ovariectomy, and is popularly termed 'hormone replacement therapy' (HRT). The usual concept implicit in the term HRT is the prescription of oestrogens to repair the major endocrine deficiency resulting from ovarian failure or ovariectomy; this implies, in biochemical terms, correcting the markedly lower plasma oestradiol levels in postmenopausal women to levels that normally circulate in premenopausal women. An alternative concept of HRT is the prescription of oestrogens specifically for the relief of complications proven to result from oestrogen deficiency in postmenopausal women. One aim of this study was to relate the biochemical effects of postmenopausal oestrogen therapy to current concepts of HRT.

Wilson and Wilson (1963) have advocated oestrogen therapy for all women after the menopause. However most clinicians have

adopted a more conservative approach, prescribing oestrogen to relieve or prevent complications definitely attributable to oestrogen deficiency after the menopause, in the lowest dose that just relieves or prevents these complications. However this dose of oestrogen is usually determined on an empirical basis. The usual doses of oral therapy that have been found to relieve specific climacteric symptoms (i.e. superficial dyspareunia and/or flushes) or prevent osteoporosis are 3mg of piperazine oestrone sulphate, 2mg of oestradiol valerate, 1.25mg of conjugated equine oestrogens, 25 µg of ethinyl oestradiol or 25 µg of mestranol (Utian, 1972; Aitken et al., 1973; Campbell et al., 1976; Gordan, 1976; Gallagher and Nordin, 1975; Jones et al., 1976; Lindsay et al., 1976 and Marshall and Nordin, 1976, 1977). These doses of postmenopausal oestrogen therapy may, however, be associated with an increased thrombogenic risk because they alter some blood clotting factors (Coope et al., 1975; Notelovitz, 1976). More importantly however, in a prospective study it was found that 36% of women prescribed these doses of oestrogen developed endometrial hyperplasia (Whitehead and Campbell, 1978). The 6-8 fold increase in the incidence of endometrial carcinoma in women prescribed postmenopausal oestrogen therapy (Ziel and Finkle, 1975; Smith et al., 1975; MacDonald et al., 1977 and Gray et al., 1977), has been attributed by Ziel and Finkle (1976) specifically to an increase in the plasma oestrone levels because most of these women were taking conjugated equine oestrogens, a mixture of oestrogens containing 50% oestrone sulphate (Stern and Givner, 1978). However Yen et al. (1975) have shown that plasma oestrone levels in

postmenopausal women increase markedly after the oral administration of oestradiol. One aim of this study was to compare the effects on plasma oestrone and oestradiol concentrations of oestrogen preparations containing either oestrone or oestradiol. Preparations containing other oestrogens such as equilin, ethinyl oestradiol or mestranol were not studied because they consist of oestrogens which do not supplement biochemically the unconjugated plasma oestrone and oestradiol produced from adrenal and ovarian sources. Moreover, they cannot be detected in the radioimmunoassays for oestrone and oestradiol.

In contrast to the effects of oral oestradiol, the vaginal administration of oestradiol (Rigg et al., 1978) or implants of oestradiol (Studd et al., 1977) preferentially increase plasma oestradiol rather than oestrone concentrations. A further aim of this study was to determine the effects of different routes of administration of oestrogen on plasma oestrone and oestradiol concentrations after the menopause, especially to determine the interrelationship between oestradiol, the more biologically potent circulating oestrogen (Fotherby, 1976), and oestrone, which Siiteri et al., 1974) have hypothesised is a possible endometrial or breast carcinogen in some genetically predisposed women.

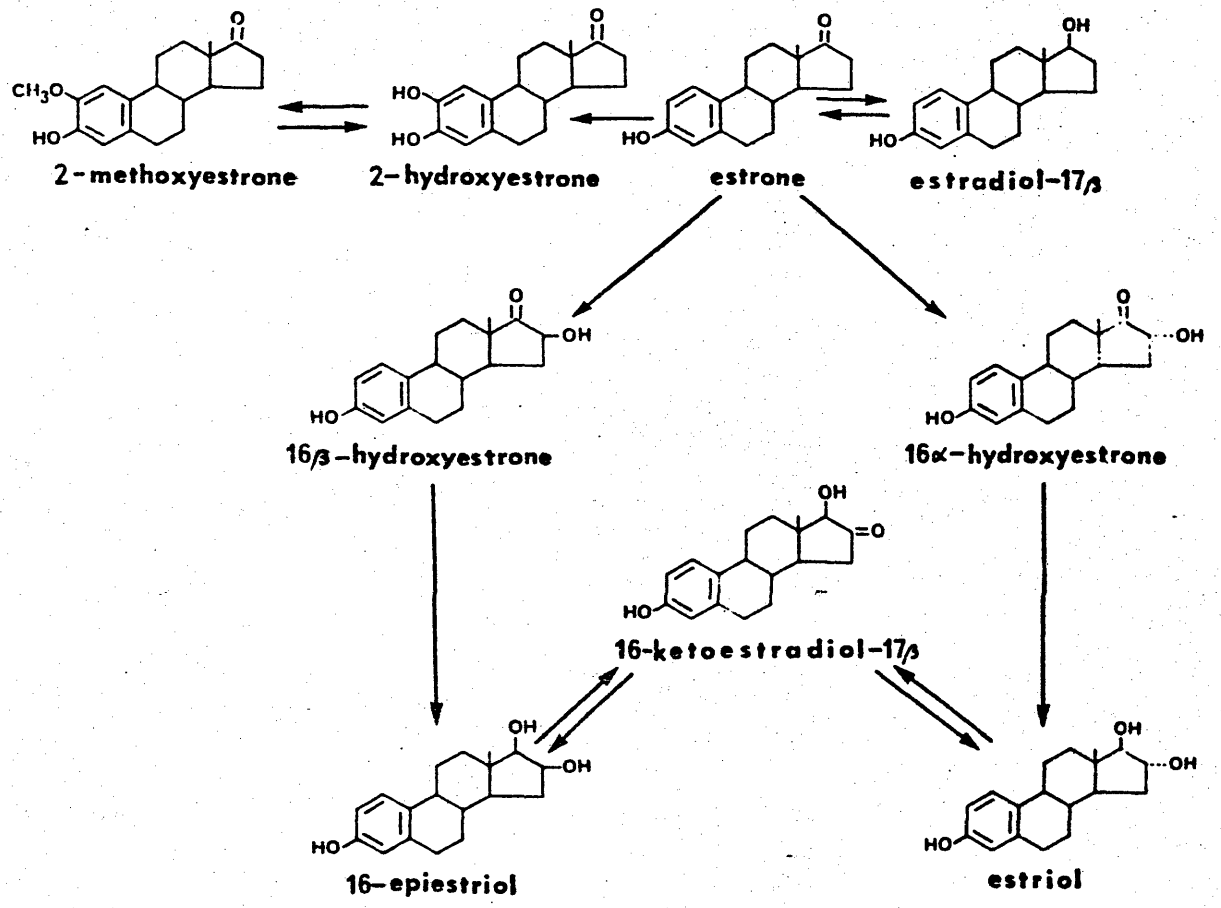
C) GENERAL CRITIQUE OF THE STUDY METHODOLOGY

This study assessed the oestrogen status of women after the menopause by determining the unconjugated plasma oestrogen concentrations, rather than by measuring excretion rates or by cytohormonal techniques because, classically, the 'dose' of oestrogens in the blood is directly related to the biological response. The determination of the excretion rate is only an indirect measure of oestrogen status because there is extensive peripheral metabolism of unconjugated plasma oestrogens, particularly in the gut and liver, before their excretion in the urine: some of the important pathways in the metabolism of oestrone and oestradiol, the major unconjugated oestrogens circulating in postmenopausal women, are shown in Figure 1.5 . Furthermore, as described previously, the reliability of cytohormonal techniques in assessing oestrogen status has not yet been widely accepted.

In many biochemical studies of plasma oestrogens after the menopause, the oestrogen determined is the sum of oestrone and oestradiol. As described previously, these interconvertible oestrogens have different biological potencies and separate pathways of peripheral production, at least in younger women. The distinction between the plasma concentrations of oestrone and of oestradiol was however possible in this study by employing highly specific antisera in the radioimmunoassays: thus, possible differences in the clinical effects and methods of production of unconjugated plasma oestrone and oestradiol were able to be accurately investigated.

Although only unconjugated oestrogens were determined in this study, conjugated oestrogens also circulate in the blood. However

Figure 1.5. Major metabolic pathways of plasma oestrone and oestradiol (from Adlercreutz, 1974).


2-methoxyestrone
2-hydroxyestrone
estrone
estradiol-17β
16β-hydroxyestrone
16α-hydroxyestrone
16-ketoestradiol-17β
16-epiestriol
estriol

being conjugated, they probably have no biological activity (Hawkins and Oakey, 1974). Quantitatively, the conjugate oestrone sulphate is the major circulating oestrogen in young women (Loriaux et al., 1971; Hawkins and Oakey, 1974), and although originally regarded as an oestrogen excretory product (McKenna et al., 1961), it is now thought to have a vital role in the physiology and metabolism of the biologically active unconjugated plasma oestrogens (Fishman et al., 1969). All circulating oestrone sulphate is probably derived from unconjugated plasma oestrone and oestradiol (Ruder et al., 1972); significant peripheral interconversion between the conjugated and unconjugated oestrogens also occurs (Longcope, 1972). Furthermore, and in contrast to the unconjugated plasma oestrogens, plasma oestrone sulphate has a long half-life of about four hours (Twombley and Levitz, 1960; Ruder et al., 1972), and a large volume of distribution (Longcope, 1972; Ruder et al., 1972). For all these reasons, plasma oestrone sulphate may be important in the metabolism of, and particularly as a source of, unconjugated plasma oestrone and oestradiol. However, oestrone sulphate was not examined in this study of plasma oestrogens after the menopause because the technique for determining its plasma concentration was not available, being more complicated and time consuming than the radioimmunoassay technique used for determining the unconjugated plasma oestrone and oestradiol concentrations.

The biological activity of unconjugated plasma oestrone and oestradiol depends only on the unbound fraction (Anderson, 1974). Oestrone circulates loosely bound to albumin whereas oestradiol is bound with higher affinity to sex-hormone-binding globulin (SHBG) whose rate of production is dependent, inter alia, on circulating

oestrogen levels (Anderson, 1974). Because of the loss of the follicular source of plasma oestrogens after ovarian failure, the unbound fraction of oestradiol, but not oestrone, is slightly higher in postmenopausal women than in premenopausal women (Table 1.1). However, because changes in plasma albumin or SHBG concentrations occur over several days, the proportion of oestrone and oestradiol that is unbound is constant, and thus the total unconjugated plasma oestrogen concentrations determined in this study are directly related to the amount of oestrogen available to effect a response.

The plasma concentration of oestrogen is directly related to the total amount of the unconjugated oestrogen being produced from all sources, and is inversely related to the metabolic clearance rate of the oestrogen, according to the classic equation:

$$\text{Plasma concentration of Oestrogen} \longrightarrow C_E = \frac{PR_E \leftarrow \text{Total Production Rate of Oestrogen}}{MCR_E \leftarrow \text{Metabolic Clearance Rate of oestrogen}}$$

In postmenopausal women, the plasma oestrogen level is directly related to the sum of the rates of four possible methods of production:

1) direct secretion by the stroma of the postmenopausal ovary
2) direct secretion by the adrenal cortex
3) peripheral production from plasma androgens and plasma conjugates such as oestrone sulphate
4) release into the circulation of oestrogens stored extravascularly, such as in fat (Twombley et al., 1967).

However, the plasma level also depends inversely on the metabolic clearance rate which is the sum of the clearance rates of unconjugated

Table 1.1. Ranges of the unbound fraction of oestrone and oestradiol in premenopausal women (during the follicular phase of the menstrual cycle) and in postmenopausal women determined by equilibrium dialysis using 1:5 diluted plasma. (Adapted from Hutton et al., 1978).

	Per cent unbound in plasma	
	Oestrone	Oestradiol
Premenopausal	8.0-12.0	3.0-5.7
Postmenopausal	7.0-13.0	4.4-6.2

oestrogens by all the tissues and organs in the body as blood flows through them. The particular clearance rate of oestrogen in an organ or tissue is a product of the organ or tissue blood flow and the 'extraction', that is the fraction of unconjugated oestrogen that is irreversibly metabolised (Tait, 1963). Thus:-

$$MCR_E = \sum \left(\begin{array}{l} \text{Each organ/tissue Blood} \\ \text{Flow x Extraction by each organ} \end{array} \right)$$

In this study of plasma oestrogens after the menopause, factors known to affect metabolic clearance rates of oestrogen, such as posture (Flood et al., 1973) were kept constant wherever possible, and thus, the production rate of oestrogens was able to be studied directly.

The contribution of the postmenopausal ovary to plasma oestrogen and androgen production after the menopause has previously been investigated by gradient techniques, and pre- and post-ovariectomy studies. However, the conclusions of gradient studies, in which ovarian vein steroid concentrations were compared with peripheral vein concentrations, made no allowance for the ovarian vein blood flow (which clinically is low after the menopause), nor possible interference of the sampling method with the ovarian secretion of steroids. Pre- and post-ovariectomy studies in which peripheral steroid concentrations immediately before ovariectomy are compared to post-ovariectomy levels make no allowance for the possibility that the stress of an operation might interfere with steroid production and metabolism. Furthermore, neither method of study has allowed for possible spontaneous fluctuations in steroid levels. For all these reasons, further studies are required to determine the exact role of the postmenopausal ovary in plasma

steroid production after the menopause.

The role of the adrenal cortex, and particularly ACTH, in the production of plasma oestrogens after the menopause has been deduced from studies of urine, and venous gradients (whose values and limitations have just been described) and of ACTH suppression and stimulation studies. This last method has been particularly valuable in exploring the regulatory role of ACTH in the production of plasma oestrogens and androgens, but only results from a few blood samples have previously been reported, and therefore the dynamics of the production has not been resolved. By suppressing ACTH secretion with dexamethasone treatment for different periods and infusing, rather than injecting , ACTH, attempts were made to confirm the conclusions of the previous studies and also to explore the dynamics of the adrenal contribution to androgen and oestrogen production after the menopause.

Much of our understanding of the production and metabolism of plasma oestrogens after the menopause has been gleaned from in vivo isotopic studies. The administration of labelled tracers does not disturb endogenous oestrogen production and has therefore been a particularly valuable method of study. However some of the concepts such as the interconversion of oestrone, oestradiol and oestrone sulphate, have developed from studies in men, young women and postmenopausal women with breast or endometrial carcinoma. Further studies in normal postmenopausal women are therefore necessary because oestrogen production and metabolism changes in relation to sex and age (Longcope et al., 1968; Siiteri and MacDonald, 1973), and may be abnormal in women with breast or endometrial carcinoma (Siiteri et al., 1974). The results of isotopic infusion studies

have usually required that steady-state conditions apply (i.e. the amount of non-isotopic steroid in the system is assumed to be constant, and the rate of entry of a steroid into a particular pool is equal to its rate of exit (Baird et al., 1969). Equilibrium conditions, especially with oestrogens in postmenopausal women, may however not always be obtained, even after several hours of infusion (Pearlman et al., 1969; Hembree et al., 1969). Therefore, other forms of study, such as by the infusion of 'cold' (non-isotopic) steroids as employed in this study are necessary to further examine the dynamics of plasma oestrogen production and metabolism, especially in the phase before equilibrium is achieved.

The recent development of the radioimmunoassay technique of saturation analysis has resulted in the concentrations of plasma steroid and gonadotrophin concentrations being easily and accurately determined in just a few millilitres of blood. Thus, intensive plasma hormone profiles, which are pictures of several hormones determined in many blood samples, have been developed. Hormone profile techniques are often complementary to in vivo isotopic techniques but do not have a radiation risk to the subjects who are studied. In this study, intensive hormone profile techniques were employed to examine the production and metabolism of oestrogens after the menopause.

It is now well known that plasma concentrations of many hormones vary throughout the day because the production of these hormones does not occur at a steady rate: thus the accurate determination of the mean plasma hormone level requires several blood samples to be obtained throughout the day. In most previous studies of oestrogen production after the menopause, the steroid hormone concentrations

have been determined in random blood samples but, Vermeulen (1976) and Campbell et al. (1976) obtained blood samples at four and two hour intervals respectively, and thus showed that plasma steroid hormone concentrations in postmenopausal women also varied throughout the day. In this study, many blood samples were obtained so that the endocrine status of women after the menopause could be accurately determined by intensive hormone profile techniques.

The half-time for the disappearance from the blood of all unconjugated steroids is less than 90 minutes and therefore fluctuations in plasma levels that result from episodic production can only be determined if a frequent blood sampling schedule, such as every 20-30 minutes, is employed. Weitzman et al. (1966) first employed such a technique of frequent sampling and thus showed that cortisol secretion by the adrenal was episodic. Subsequent studies have since demonstrated that the diurnal and episodic secretion by the adrenal of cortisol, a unique adrenal product, and of androstenedione are both regulated by ACTH (Berson and Yalow, 1968; Tunbridge et al., 1973). Fluctuations in cortisol levels can therefore be regarded as reflecting ACTH stimulation of the adrenal. Episodic, but not diurnal, production of other hormones important to this study (oestrone, oestradiol, testosterone FSH and LH) has also been demonstrated in men by these intensive hormone profile techniques (Yen et al., 1972; Alford et al., 1973; de Lacerda et al., 1973; Bodenheimer et al., 1973; West et al., 1973 and Leymarie et al., 1974). This study employed similar sampling techniques to determine if the production of steroids in postmenopausal women was also episodic. Furthermore, the

adrenal, ovarian and extraglandular production and metabolism of oestrogens after the menopause were also studied by examining the interrelationships of the hormone concentrations in the intensive plasma profiles.

D) METHODS

(1) CLINICAL

The general details of the patients studied, the sampling and infusion techniques and the drugs administered are described here: other details are specified in subsequent sections as appropriate. The approval of the St. Mary's Hospital Ethical Committee was obtained prior to the commencement of the study.

Patients: All patients recruited to this study were attending or referred either to the menopause clinic because of climacteric symptoms, or to the gynaecological clinic for consultations or postoperative follow-up checks. Except where specified, none of the patients had a hormone dependent tumour or had taken drugs or hormone preparations for at least four months prior to the study. The individual clinical details are included together with all the biochemical results for each patient in the appendix: clinical details of the various groups of women studied are specified in the relevant sections. The non-ovariectomised women, whose ages ranged from 49-62 years, were considered postmenopausal because they had a history of amenorrhoea for at least 0.4 years (mean 2.8; range 0.4-7.0) and at the time of recruitment, their plasma gonadotrophin levels were raised into the range found in primary ovarian failure (Jacobs and Murray, 1976).

After a full disscussion of the aims of the study and the methods employed, patients who consented to inclusion in the research project were admitted to hospital for hormone profile studies at times that were convenient to them. In the first half of the study, patients were admitted to the Samaritan Hospital but subsequently they were admitted to the metabolic ward at

St. Mary's Hospital where the nursing staff assisted in collecting the blood samples. In patients prescribed glucocorticoid or oestrogen therapy as outpatients, the sampling began shortly after their admission, whereas the sampling in the other women began after several hours rest (usually overnight) in hospital. Except for toilet requirements, the women rested in bed where their posture was usually, but not always, recumbent. The patients had their meals at about 0800, 1200 and 1730 hours with light refreshments at about 0630, 1430 and 2000 hours.

Sampling technique: Materials used in obtaining the blood samples are detailed in Appendix I. In the intensive hormone profile studies, a cannula with a three-way stopcock attached, was introduced into a forearm vein immediately prior to the commencement of sampling. After withdrawing and discarding the first 1ml, 5ml blood samples were obtained at 20-30 minute intervals. The cannula was flushed with 1-2 ml of heparinised saline (100 in/ml) so that it would not become obstructed by a clot. After mixing in heparinised tubes the blood samples were centrifuged at 3,000 rpm for five minutes. The plasma was then separated using Pasteur pipettes and stored at -20°C in labelled tubes until required for assay.

Infusion procedure: The materials and drugs used for the infusion studies are described in Appendix II. Physiological amounts of the hormones were infused in acid or protein solutions (to prevent their adsorption to glass) over a six hour period. The amount of tetracosactrin infused was determined as the lowest dose rate which evoked a maximal adrenal response, i.e. 3µg/hr (Landon et al., 1964). The amount of steroid

infused in the six hours was approximately the mean daily production rate for postmenopausal women: this was calculated as the product of the mean plasma steroid concentrations (as determined from the results in the normal postmenopausal and ovariectomised women from whom blood samples had been obtained every 20 minutes for 24 hours) and the metabolic clearance rate (using values in the literature obtained by isotopic techniques). All values used in calculating the amount of steroid infused in this study are shown in Table 1.2 .

Stock ethanolic solutions of each steroid hormone were prepared at an appropriate dilution from weighed amounts of powdered steroid, and stored at 4°C. Immediately prior to each infusion, a 50ml solution of the hormones to be infused was prepared aseptically in sterile glassware and, after thorough mixing, drawn into a syringe. This syringe was then attached to an electrically driven pump and the solution delivered at a constant rate of 6.7ml/hr. A scalp-vein needle was then introduced into a vein on the dorsum of the hand or arm of the opposite limb from which the blood samples were being obtained. During the six hour infusion, the patients were always supine, but food was not usually withheld.

Infusion solutions:

Tetracosactrin: 22.5ug (0.1ml) was mixed with 50ml of acidified saline and thus, when infused, the rate was 3µg/hr.

Androstenedione: 1.9 ml of an ethanolic solution of androstenedione (1mg/ml) was mixed with 8.1ml of normal saline which was then added to 40ml of an albumin solution: thus, the infused rate was 0.25mg/hr.

Androstenedione and cortisol: 1.9ml of the stock solution of

Table 1.2. Values used for determining the amount of steroid to be infused and the total amounts actually infused. The mean plasma concentrations were determined from the results of intensive hormone profile studies in normal postmenopausal and ovariectomised women of similar age and weight to the infusion subjects.

Steroid	Mean plasma Concentration	Metabolic Clearance l/day	reference	Calculated Daily Production Rate	Actual amount Infused (6hr)
Androstenedione	82 ng/dl	1850	Abraham et al., 1969	1.5 mg	1.5 mg
Testosterone	32 ng/dl	760	Gower, 1975a	240.0 µg	290.0 µg
Cortisol	8 µg/dl	200	Gower, 1975a	16.0 mg	15.0 mg
Oestradiol	3.7 ng/dl	1400	Longcope et al., 1968	52.0 µg	60.0 µg
Oestrone	5.5 ng/dl	2200	Longcope et al., 1978	121.0 µg	120.0 µg

androstenedione was mixed with 1.9ml of cortisol (10mg/ml) and 6.1ml of normal saline before adding to 40ml of the albumin solution: the infusion rates of androstenedione and cortisol were therefore 0.25 mg/hr and 2.5mg/hr respectively.

Testosterone: 1.2ml of stock solution (0.3mg/ml) were mixed with 8.8ml of saline and then added to 40ml of the albumin solution:so that the infusion rate was 48µg/hr.

Oestradiol: 0.75ml of stock solution of oestradiol (100ug/ml) was mixed with 9.25ml of saline and then added to 40ml of albumin: the infusion rate was therefore 10µg/hr.

Oestrone: 1.5ml of the oestrone stock solution was mixed with 8.5ml of saline and 40ml of albumin, so that the infusion rate was 20µg/hr.

(2) LABORATORY

The plasma concentrations of all hormones studied were determined by saturation analysis techniques developed and established in either the Department of Chemical Pathology under the supervision of Professor V.H.T. James or the Department of Obstetrics and Gynaecology under the supervision of Dr. H.S. Jacobs and Dr. M.A.F. Murray. The principles of the techniques of saturation analysis and radioimmunoassay have been well reviewed by Ekins and Newman (1970) and others. Radioimmunoassay is a quick and inexpensive method of reliably determining the concentration of hormones in many samples using only minute amounts of plasma. The radioimmunoassay method for determining the plasma concentrations of oestradiol-17β is fully described below because all the determinations in this study were performed by the author. Radioimmunoassay methods for

determining the plasma concentrations of the other hormones are described only briefly because they were necessarily performed by other persons using materials, methods and instruments which were often similar to those employed in the oestradiol assay. In this study the hormone concentrations from one woman were always determined in a single assay, but when the value of a concentration in a single sample was doubted, the value was confirmed by repeating the determination in a subsequent assay.

Oestradiol Radioimmunoassay

The materials, radiolabelled oestradiol solutions and the methods of sample storage, solvent evaporation, centrifugation, mixing, shaking and incubation, and of the preparation of the gelatin buffer, charcoal suspension, toluene scintillant and glassware are described in Appendix III.

Assay method: The assay method was developed by Dr. M.A.F. Murray based on that described by Hotchkiss, Atkinson and Knobil (1971). The volume of plasma samples, water blanks and plasma pools employed in this assay was 0.7ml when very low levels of oestradiol were anticipated, such as in the dexamethasone suppression studies; otherwise 0.5ml of plasma was employed. This volume was pipetted into 6ml stoppered tubes, after which 50µl (about 1,000 cpm) of ^{3}H-oestradiol recovery solution was added for the later correction of extraction losses. This volume of recovery fluid was also added to three counting vials and, after evaporation, 10ml of toluene scintillant and 100µl of gelatin buffer were added so that the total recovery counts could be determined. After gentle mixing of the recovery fluid and the plasma samples, and ten minutes incubation, 5ml of

diethyl-ether was added to each tube, a fresh bottle of ether being opened for each assay. The samples were then extracted by shaking for 15 minutes after which the tubes were left for several minutes to allow separation of the plasma and solvent layers. The plasma layer was then frozen by placing the tubes in a mixture of dry-ice and acetone. The solvent layer was then poured off into another set of tubes. The ethereal solution was evaporated and redissolved in 400µl of the gelatin assay buffer: 200µl of this extract was then taken for the assay, and a further 100µl was added to counting phials containing 10ml of the scintillant for correcting for the losses during the extraction.

Tubes containing 0,5,10,20,30,50,100 and 150pg of oestradiol-17β were prepared from an ethanolic solution of oestradiol (1pg/µl) in triplicate (together with total count tubes) for the standard curve. 20µl of recovery fluid was added to each tube for the standard curve, and the solution was then evaporated and redissolved in 200µl of gelatin buffer.

100µl of the antiserum solution was then added to the 200µl solutions in the assay and standard curve tubes. This antiserum was kindly supplied by Dr. G. Knaggs: it had been prepared by injecting emulsified oestradiol-17β-6-keto-bovine serum albumin conjugate (prepared by the method of Erlanger et al., 1957) into a goat (no. 510). The plasma obtained on 17th January, 1974 was treated as described by Exley et al. (1971) and then diluted 1:100 in the gelatin buffer. 200ul aliquots were then stored at -20°C until required for the assay. The antiserum had been serially diluted and the % binding of 40pg of ^{3}H-oestradiol was determined over the range 1:4,000-1:16,000; the concentration selected

(1:10,000) bound 40-50% of the labelled oestradiol. After gentle mixing, the tubes containing the antiserum were incubated at room temperature for 30 minutes. Then 100µl of radioactive buffer (10,000 cpm; approx. 40pg ^{3}H-oestradiol) were added, and the tubes again gently mixed. After a further 30 minute incubation period, the tubes were transferred to pre-cooled containers in a cold tray. 1ml of a refrigerated charcoal suspension was then added to all tubes, except the total count tubes to which 1ml of gelatin buffer was added. After thorough mixing, all tubes were left in the cold tray for 15 minutes and then transferred to cooled centrifuge carriers and centrifuged at 3,000 rpm for 3 minutes at 4°C. The supernatant, containing the antibody-bound labelled hormone, was then decanted directly into counting phials that contained 10ml of the toluene scintillant. After shaking for 10 minutes, the phials were counted for 10 minutes or to 10,000 counts whichever occurred the sooner.

Calculations: The standard curve was constructed by plotting the time to reach 10,000 counts as a function of the mass of unlabelled oestradiol. This gave a straight line as shown in Figure 1.6 . The values for the assay samples were then visually interpolated. By correcting for extraction losses, the plasma concentration in pg/ml was determined for each sample by using the equation:

$$\text{concentration} = \frac{\text{pg/assay tube}}{\text{recovery (cpm)}} \times K$$

where the correction factor K = :-

$$\frac{\text{mean total recovery counts (cpm) x volume of recovery extract (µl)}}{\text{volume of sample (ml) x volume of assay extract (µl)}}$$

Reliability criteria: The reliability criteria for the oestradiol assays performed by the author were:

i) Recovery: The mean recovery of ^{3}H-oestradiol added to 50 plasma samples per assay, and in 40 assays was 82 $\pm$ 7 (SD)%.

ii) Standard curve: A typical standard curve is shown in Figure 1.6 where each point represents the mean of replicates. The slope $(\frac{x}{y})$ of the standard curves in the study as determined graphically ranged from 1.1-1.4 . When the binding of 40pg of ^{3}H-oestradiol was $<$ 30%, as sometimes occurred in warm weather, the assay was rejected.

iii) Accuracy: The closeness of measurements to the true value was determined by adding known 10-50pg amounts of standard oestradiol to water and ether extracted plasma (Mayes and Nugent, 1970), and the amount recovered by the usual assay method was within 10% of the theoretical value. The plasma concentrations of four samples in the range 5-40ng/dl were also determined by mass spectrometry by the Tenovus Institute, Cardiff: the values of all samples determined by radioimmunoassay were 15-17% lower than those determined by mass spectrometry.

iv) Precision: The variation of replicate estimates was determined by the inter-assay and intra-assay variations of oestradiol concentrations of plasma pools (Table 1.3).

v) Sensitivity: Good sensitivity was vital in this study, especially because many of the values calculated in the assays ranged between 0-10pg/tube. Two standard deviations of the per cent free above the 5pg standard could always be distinguished from two standard deviations below that of the

Figure 1.6. Typical standard curve of oestradiol in the range 0 -150 pg.

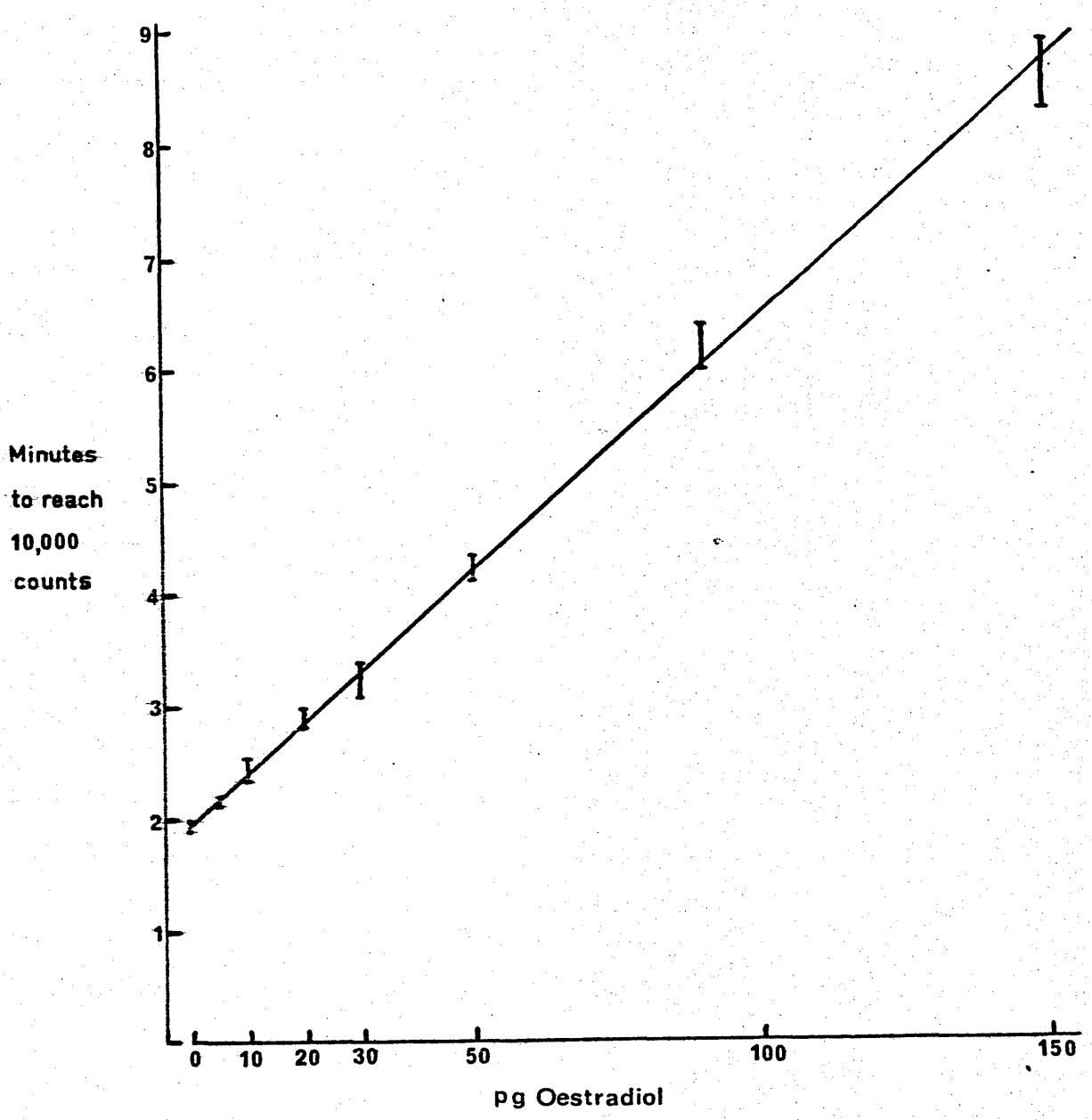

Minutes to reach 10,000 counts
9
8
7
6
5
4
3
2
1
0
10
20
30
50
100
150
pg Oestradiol

Table 1.3. Precision: the results of replicate estimations of oestradiol in plasma pools from males and females. Mean results ng/dl ± SD are given. CV = coefficient of variation, n = the number of analyses.

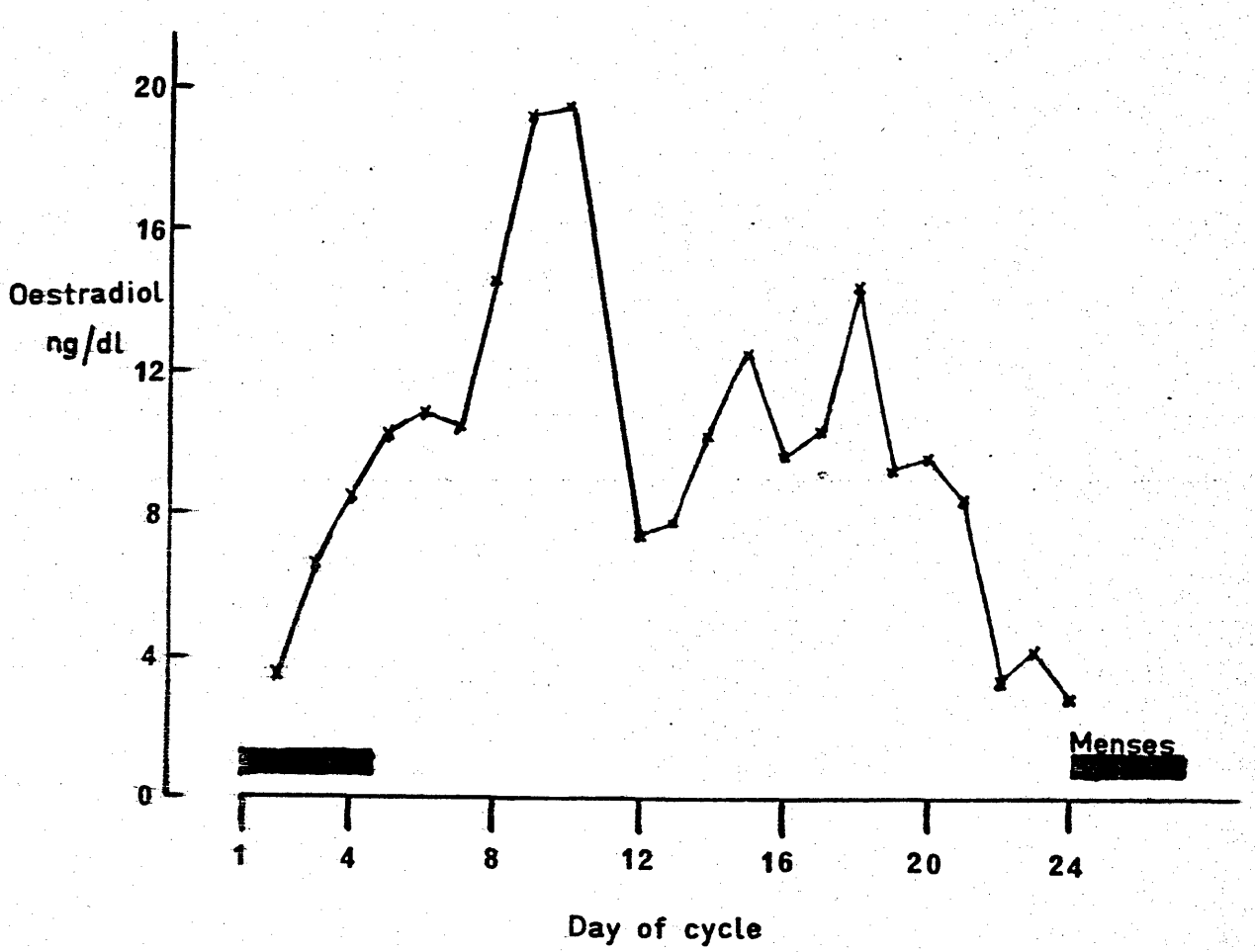
Pool	Intra-assay variation	CV %	n	Inter-assay variation	CV %	n
Male 1	3.9 ± 0.32	8.2	6	4.8 ± 0.61	12.6	33
Male 2	8.0 ± 0.61	7.7	11	-	-	
Female 1	22.3 ± 1.68	7.5	16	16.3 ± 1.80	11.1	43
Female 2	-	-		7.0 ± 0.81	11.5	46

zero standard. When sensitivity was determined according to the method of Midgley et al. (1969), the smallest amount that differed significantly from zero, when calculated graphically from the standard curve by taking standard deviations from replicate zero estimations, was usually 2pg/tube, and was never more than 4pg/tube: after correction for the mean percentage recovery of the assay system, and the original volume, the limit of sensitivity of the method was usually 1ng/dl, and never more than 2ng/dl.

vi) Specificity: The specificity of the 6-keto-BSA antiserum had been previously determined by Dr. Murray: the cross reactions of the antiserum with all steroids in competition with oestradiol-17β, including conjugates were all less than 1%, and were often undetectable when calculated by the method of Abraham (1969). In the assays performed by the author, distilled water blanks were undetectable except on four occasions when water blanks gave values $>$1.5ng/dl - these assays were rejected. Plasma from gonadectomised, adrenalectomised subjects was not available for assay. The oestradiol concentration of plasma extracts which were chromatographed on paper were identical to those determined without a chromatographic step (Murray, 1975). Finally, the plasma oestradiol levels in daily blood samples obtained from a 40 year old woman who had a 24 day menstrual cycle (Figure 1.7) are comparable to those reported by others using similar techniques (Dufau et al., 1970; Abraham et al., 1970; Ribeiro et al., 1974 and Sherman et al., 1976).

It was concluded from the experiments described above that the

Figure 1.7. Plasma oestradiol concentrations in daily blood samples from a 40 year old woman during one menstrual cycle.


20
16
Oestradiol
ng/dl
12
8
4
0
Menses
1
4
8
12
16
20
24
Day of cycle

plasma oestradiol-17β concentrations in postmenopausal women to be described, were determined with acceptable reliability.

Cortisol, Androstenedione, Testosterone and Oestrone radioimmunoassays

The plasma concentrations of these steroids were determined by radioimmunoassay techniques using specific antisera (James et al, 1977; Reed et al, 1979; Goodall et al, 1979). In the assays, the cross-reactivity of each steroid with other steroids known to circulate in detectable quantities in humans, was always < 1.0% except for 11β-hydroxyandrostenedione with androstenedione. Brief details of the methods are shown in Table 1.4. The recovery of oestrone during the extraction procedure was 81 ± 8(SD)% and in each sample this was corrected by the addition of an internal standard. The reliability criteria for all these steroid assays were also acceptable for this study of plasma oestrogens after the menopause.

FSH and LH Radioimmunoassays

Plasma FSH and LH concentrations were determined using specific antisera provided by Dr. W.D. Odell and reference preparations FSH (68/39) and LH (68/40) provided by the National Institue for Biological Standards and Control of the United Kingdom (Jacobs and Lawton, 1974). The normal range of concentrations in premenopausal women are 5-50 μg/L for FSH and 0.5-2.0μg/L for LH. In postmenopausal women, the levels are increased by a factor of about five; thus, in this study the amount of plasma used for determining the concentration was one-fifth that normally used, so that the values fell in a satisfactory part of the standard curve. The intra-assay and inter-assay coefficients of variation were 5% and 10% respectively for FSH, and 6% and 10% respectively for LH. Other reliability criteria were found on testing to also be acceptable for this study of plasma oestrogens after the menopause.

Table 1.4. Brief details of the radioimmunoassay techniques used for the determination of plasma cortisol, androstenedione, testosterone and oestrone concentrations. CV = coefficient of variation, BSA = bovine serum albumin, * = mid-follicular phase of the ovulatory cycle.

Steroid Assayed	Extraction Solvent	Antigen	Antiserum Source	Limit of Sensitivity	Assay CV % Intra	Inter	Normal Range premenopausal
Cortisol	Dichloroethane	21-hemisuccinate	This laboratory	1 µg/dl	5.0	5.0	2 - 15 µg/dl
Androstenedione	Hexane:ether	11-hemisuccinate	Dr W. Shopman (Rotterdam)	25 ng/dl	3.2	10.5	20 -250 ng/dl
Testosterone	Benzene : Pet. ether	7-BSA	Miles Research Products	10 ng/dl	7.0	10.0	15 - 70 ng/dl
Oestrone	Hexane:ether	6-(O-carboxy)	Dr D. Exley (U.K.)	1 ng/dl	4.5	7.0	*6 - 12 ng/dl

(3) STATISTICAL

Mean values in the text are usually coupled with the standard deviation (SD) and the n value. When the standard error of the mean (SEM) is employed, this is clearly stated.

The significance of differences between groups was analysed by the Student's t-test when the values were normally distributed (by variance ratio testing about the mean); when the values were not normally distributed about the mean, the statistical significance of group differences was determined by the Mann-Whitney U test. The probability (p) values expressed in the text were obtained from standard reference tables.

When the degree of correlation was determined, Pearson's correlation coefficient test was employed when the values were normally distributed and the r and p values presented; when the values were not normally distributed, Spearman's rank correlation coefficient test was used and the ρ and p values given. Confidence limits in regression figures were placed at two standard errors of the estimate ($S_y = sd_y \sqrt{1 - r^2}$)

PART 2

Summary: The purpose of these physiological studies of plasma oestrogens after the menopause have been described in Part 1. In Part 2, the investigation of the plasma hormone status of postmenopausal women is described first. Subsequently, studies of the contributions of the postmenopausal ovary and adrenal cortex to plasma steroid concentrations, and the role of extraglandular tissues in oestrogen production are described.

Part 2 a) THE PLASMA HORMONAL STATUS OF POSTMENOPAUSAL WOMEN

As described in Part 1, the plasma steroid concentrations in all previous studies have been determined in single, or only a few blood samples obtained from a small number of women of varying age, whose weight and height have not usually been specified. Allowances for possible fluctuations in hormone levels during the day have not usually been made and interrelationships between the various plasma hormone levels have not been examined in detail. In this study, the hormone profile techniques previously described were employed to determine accurately plasma hormone concentrations in normal postmenopausal women who were about of/ideal weight for their age and height, and who were within a few years of the menopause. The objective was to determine as accurately as possible the hormonal deficiencies resulting from ovarian failure. The production of oestrogens after the menopause was also investigated by examining interrelationships of plasma oestrogen concentrations with the plasma concentrations of androgenic prehormones and gonadotrophic hormones.

METHOD

After an overnight rest in hospital, four women (Subject nos. 1,2,3 & 4) whose menopause had occurred 3-5 years earlier, were studied by obtaining blood samples every 20 minutes for 24 hours beginning at 0800h. FSH, LH, oestrone, oestradiol, testosterone, androstenedione and cortisol concentrations were determined in all these samples. Two of the women (Subject nos. 1 & 2) had been referred to the menopause clinic whereas the other two women had been attending the gynaecological clinic. Subject no. 4 had an asymptomatic carcinoma-in-situ of the cervix that was incompletely

excised by cone biopsy. Two days after the study, total abdominal hysterectomy and bilateral ovariectomy was performed. No primordial follicles were identified in any of the many histological sections which were prepared of the ovaries (Anderson, 1978).

In a further 12 postmenopausal women (Subject nos. 5-16) who had amenorrhoea for 0.4-8.0 years, blood samples were collected every 20 or 30 minutes for between 4-24 hours. Plasma testosterone, oestrone and oestradiol concentrations were determined in these samples. The sampling occurred at different times of the day and the plasma levels of cortisol and androstenedione were not measured because of the diurnal rhythm in the secretion of these steroids.

The complete clinical and sampling details of the 16 postmenopausal women are given in Appendix IV. Their mean age was 52.4 ± 3.7 (SD) years and the mean duration of amenorrhoea was 2.9 ± 2.0 (SD) years. The women whose mean weight was 59.3 ± 6.1 (SD) Kg were all within 12% of their ideal weight for their height and age (Geigy, 1970).

RESULTS

The hormone profiles of the four postmenopausal women from whom blood samples were obtained every 20 minutes for 24 hours are shown in Figures 2.1, 2.2, 2.3 & 2.4, and their mean hormone concentrations in Table 2.1. Plasma cortisol levels in the four women showed the expected diurnal rhythm with peak values at about 0800h and the nadir at about 2400h. In two women (Subject nos. 1 & 2, Figures 2.1 and 2.2 respectively) there were also marked increases in the levels at about 1300h which, in one woman (Subject no. 2), was associated with an episode of vomiting. Androstenedione concentrations were, like those of cortisol, within the range seen in premenopausal women and, in each case, the pattern also was

Figure 2.1. Plasma steroid and gonadotrophin concentrations in blood samples obtained every 20 minutes for 24 hours from a postmenopausal woman (Subject No. 1).

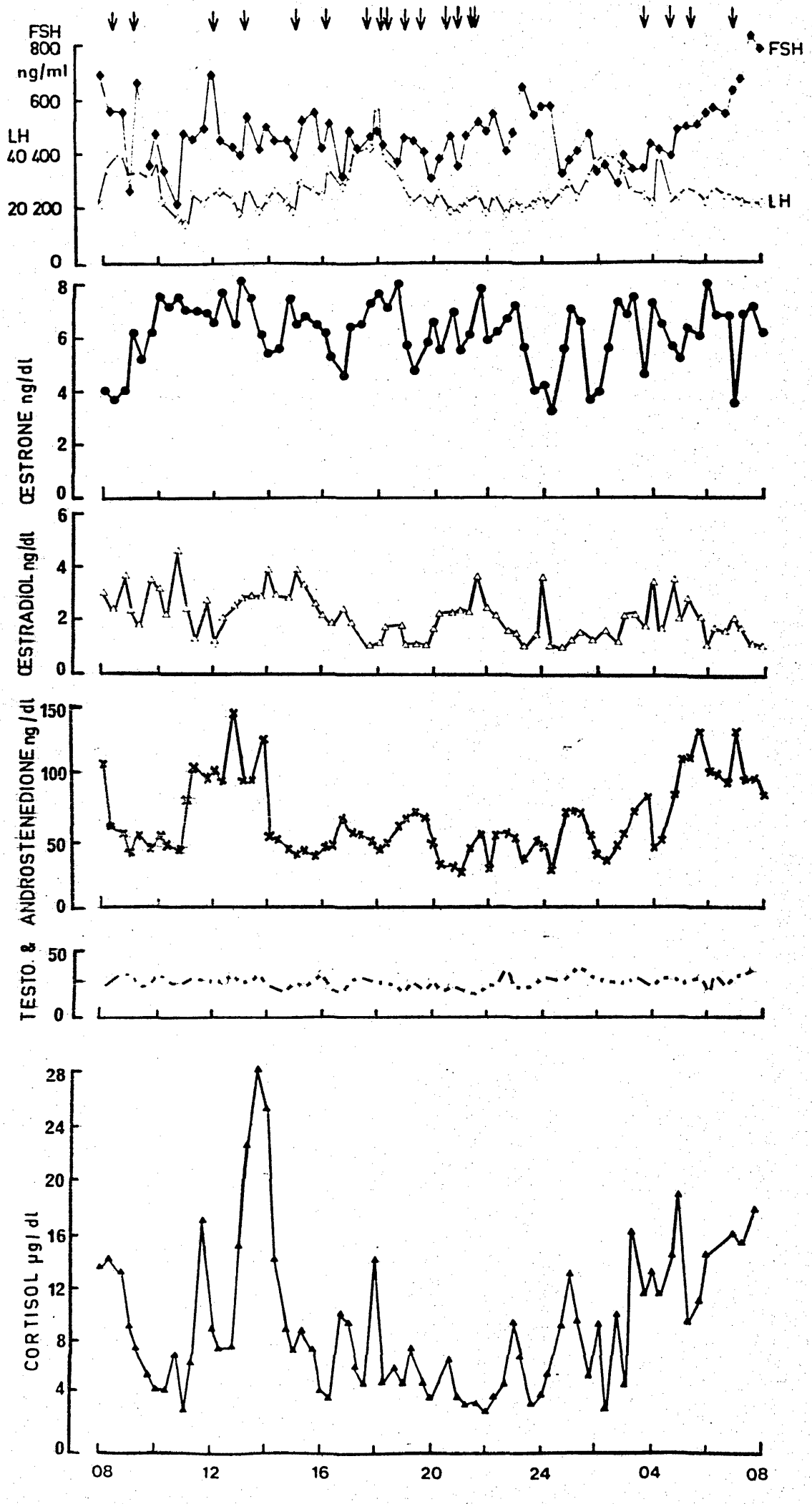

FSH
800
ng/ml
600
LH
40 400
20 200
0
FSH
LH
8
6
4
2
0
ŒSTRONE ng/dl
6
4
2
0
ŒSTRADIOL ng/dl
150
100
50
0
ANDROSTÉNEDIONE ng/dl
50
0
TESTO. &
28
24
20
16
12
8
4
0
CORTISOL µg/dl
08
12
16
20
24
04
08

Figure 2.2. Plasma steroid and gonadotrophin concentrations in blood samples obtained every 20 minutes for 24 hours from a postmenopausal woman (Subject No. 2).

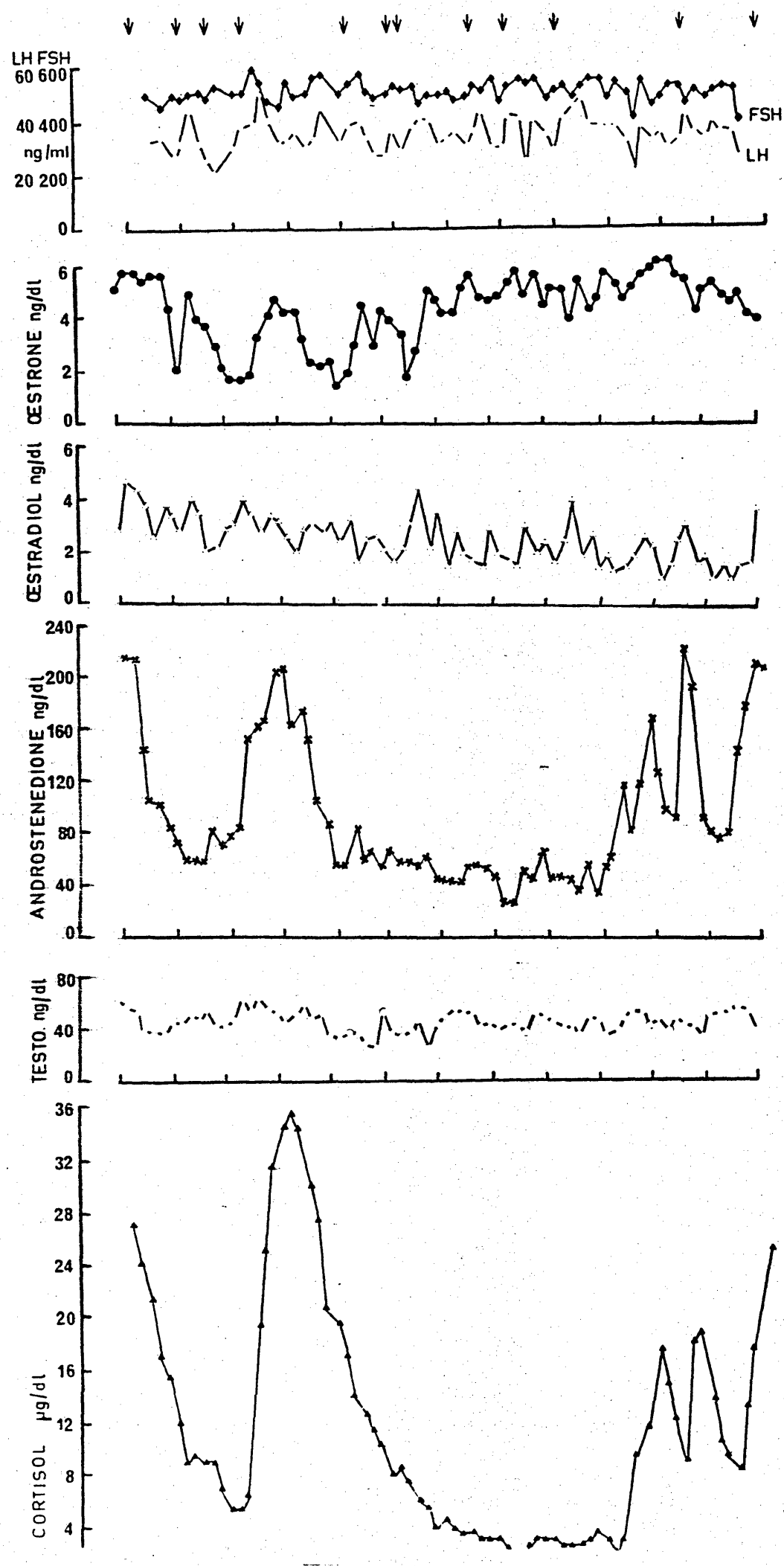

LH FSH
60 600
40 400
ng/ml
20 200
0
FSH
LH
ŒSTRONE ng/dl
ŒSTRADIOL ng/dl
ANDROSTENEDIONE ng/dl
TESTO. ng/dl
CORTISOL µg/dl

Figure 2.3. Plasma steroid and gonadotrophin concentrations in blood samples obtained every 20 minutes for 24 hours from a postmenopausal woman (Subject No, 3).

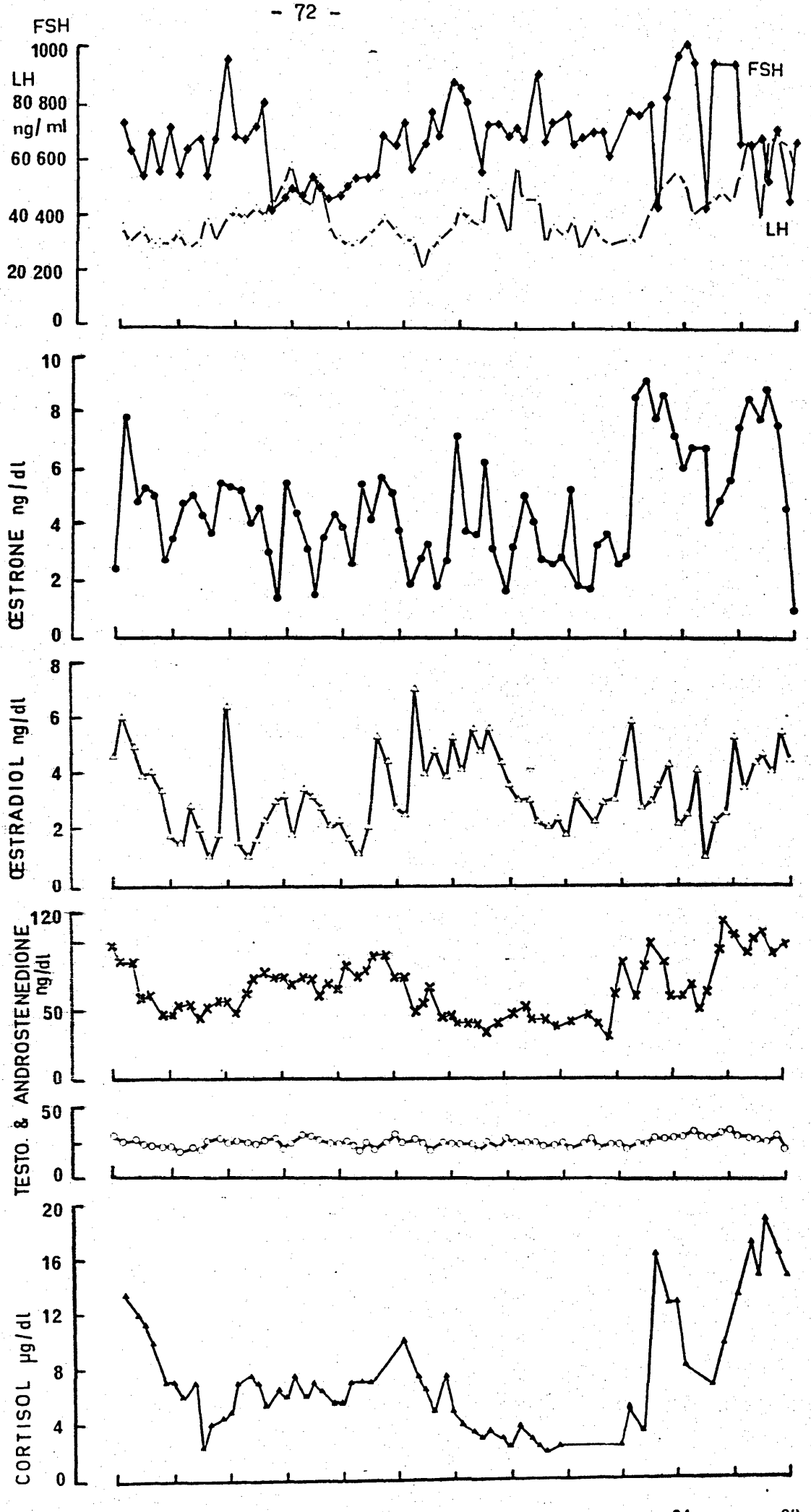

FSH
LH
ng/ ml
1000
80 800
60 600
40 400
20 200
0
FSH
LH
ŒSTRONE ng/dl
10
8
6
4
2
0
ŒSTRADIOL ng/dl
8
6
4
2
0
TESTO. & ANDROSTENEDIONE ng/dl
120
50
0
50
0
CORTISOL µg/dl
20
16
12
8
4
0
08
12
16
20
24
04
08

Figure 2.4. Plasma steroid and gonadotrophin concentrations in blood samples obtained every 20 minutes for 24 hours from a postmenopausal woman (Subject No. 4).

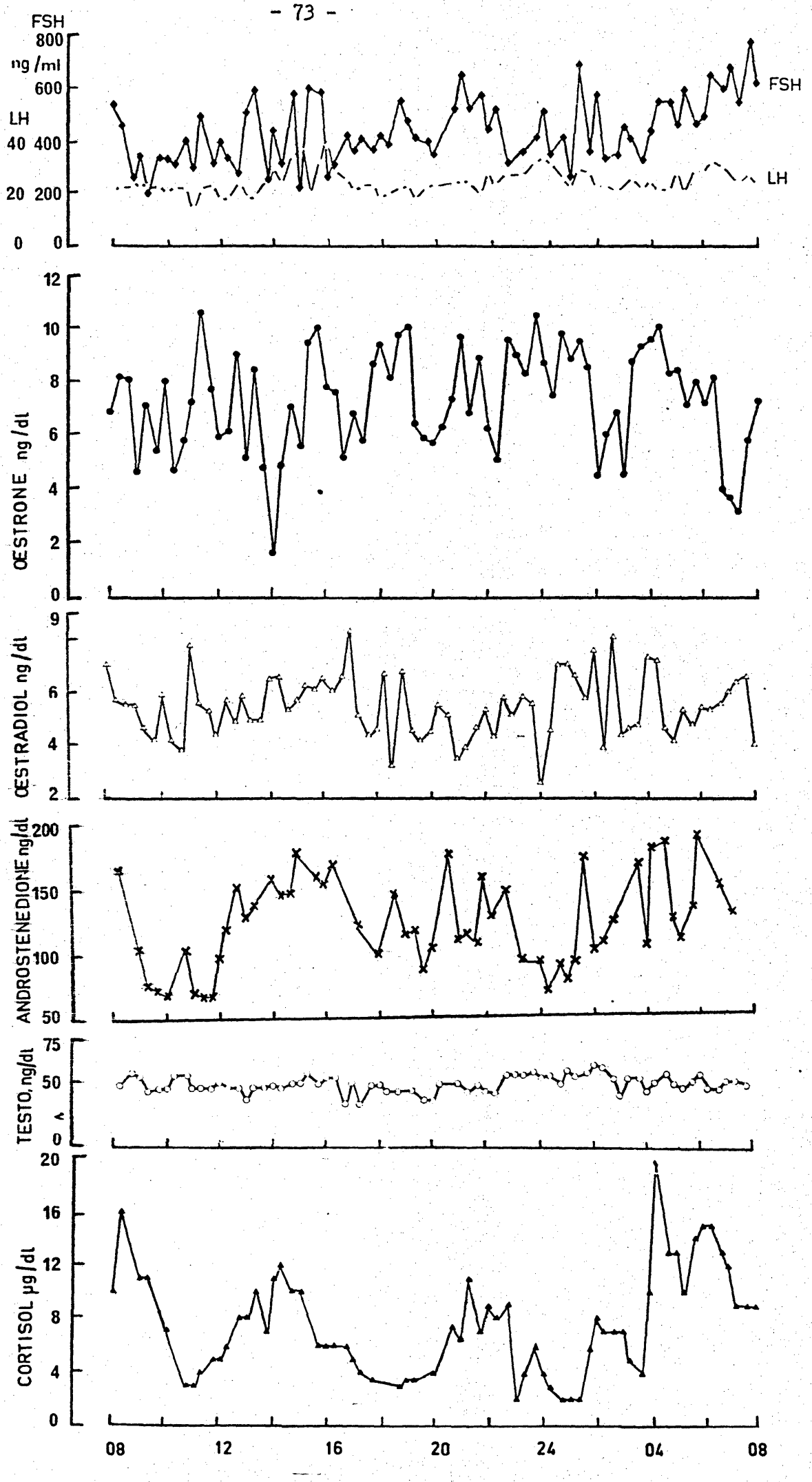

FSH
800
ng/ml
600
LH
40 400
20 200
0 0
FSH
LH
12
10
8
6
4
2
0
ŒSTRONE ng/dl
9
6
4
2
ŒSTRADIOL ng/dl
200
150
100
50
ANDROSTENEDIONE ng/dl
75
50
0
TESTO, ng/dl
20
16
12
8
4
0
CORTISOL µg/dl
08
12
16
20
24
04
08

Table 2.1. Mean ± SD of plasma hormone concentrations determined in 72 blood samples obtained every 20 minutes for 24 hours from four postmenopausal women (Subject Nos. 1-4) and two ovariectomised women (Subject Nos. 17 & 18).

NO	F µg/dl	A ng/dl	T ng/dl	E_1 ng/dl	E_2 ng/dl	LH µg/L	FSH µg/L
1	9.4 ± 5.5	66.0 ± 28.0	25.3 ± 4.4	6.1 ± 1.2	2.1 ± 0.9	26.7 ± 7.5	468.7 ± 114.8
2	11.8 ± 9.1	94.4 ± 56.2	45.0 ± 8.6	4.3 ± 1.3	2.5 ± 0.9	35.0 ± 6.1	509.6 ± 36.9
3	7.6 ± 3.9	120.5 ± 34.6	47.8 ± 6.5	7.3 ± 2.0	5.5 ± 1.2	23.5 ± 5.1	431.2 ± 125.2
4	7.3 ± 4.1	64.0 ± 21.0	26.2 ± 4.1	4.6 ± 2.0	3.4 ± 1.4	41.2 ±11.2	680.3 ± 145.3
17	5.1 ± 3.5	93.8 ± 51.9	32.9 ± 6.4	6.4 ± 3.2	4.0 ± 1.4	29.1 ± 5.9	501.4 ± 149.1
18	6.2 ± 4.3	48.6 ± 17.9	18.4 ± 6.2	4.6 ± 1.0	4.9 ± 1.0	28.7 ± 4.3	402.9 ± 89.9

similar to that of cortisol. No diurnal rhythm was detected for any of the other hormones. Plasma testosterone concentrations were also within the normal premenopausal range and showed only slight fluctuations throughout the 24 hour sampling period.

Plasma oestradiol levels showed episodic fluctuations which were not obviously associated with fluctuations of any of the other hormones. The mean oestradiol concentration for the 24 hour sampling period was 3.2 ± 1.5 (SD) ng/dl which is equivalent to the concentration in premenopausal women in the very early follicular phase of the ovulatory cycle. Compared with oestradiol, plasma oestrone levels appeared to show more marked fluctuations which were not temporally associated with those of the other hormones and particularly of androstenedione. The mean oestrone concentration in the four women - 5.6 ± 1.4 (SD) ng/dl - was similar to early to mid-follicular phase levels in premenopausal women.

Plasma FSH and LH levels also fluctuated markedly during the 24 hour sampling period. The fluctuations were not temporally associated with fluctuations in the levels of any of the steroid hormones. The mean concentrations of FSH and LH were 522 ± 102 (SD) µg/L and 32.5 ± 7.4 (SD) µg/L respectively.

The hormone concentrations in the profiles of the other 12 women studied were similar to those described above, and in particular the independent fluctuations of the plasma oestradiol, oestrone and testosterone concentrations were just as apparent as those shown for Subject nos. 1-4 in Figures 2.1-2.4. To assess the possible effect that fluctuations in plasma hormone levels might have had on the measurement of hormonal status, the accuracies

of different sampling procedures in estimating oestrone, oestradiol and testosterone status of the 16 postmenopausal women were determined.

Figures 2.5 and 2.6 show that the error in the prediction of the mean plasma oestrogen concentrations from the concentrations in the first blood sample are 2.8 ng/dl and 1.8 ng/dl for oestrone and oestradiol respectively. within their respective ranges of 1.5-8.5 ng/dl and 1.5-5.5 ng/dl. In six of 16 women in this study, the sampling period was only four hours, during which nine blood samples were obtained. The accuracy of the mean oestrone and oestradiol concentrations determined in nine blood samples in predicting the mean level in many more samples was determined from the correlations of mean oestrone and oestradiol concentrations in the other 10 women, as shown in Figures 2.7 and 2.8 respectively. In these 10 women, the possible error (within the 95% confidence limits) in the prediction of the mean level in 24-72 blood samples from the mean level in the first nine blood samples was 1.5 ng/dl and 0.6 ng/dl for oestrone and oestradiol respectively. For the purposes of this study, the error in the mean oestrogen concentrations determined in at least nine blood samples was considered acceptable, provided the mean oestrone and oestradiol concentrations were above 3 ng/dl and 2 ng/dl respectively.

Random fluctuations in the plasma testosterone levels in Subject nos. 5-16 were also similar to those shown in Figures 2.1-2.4 for subjects nos. 1-4. As shown in Figure 2.9, the possible error (within the 95% confidence limits) in the prediction of the mean concentration of testosterone for the sampling period, from the concentration in the first blood sample was 11 ng/dl.

Fig. 2.5. Correlation of plasma oestrone concentration in initial blood sample (y axis) and mean value in 9-72 blood samples (x axis) in 16 postmenopausal women (●) to show the possible error of a single oestrone determination in predicting the mean plasma oestrone concentration in blood samples obtained every 20-30 minutes for 4-24 hour. The control limits (---) are placed at two standard errors of the estimate S_y from the regression line (——) for the postmenopausal women. The oestrone values (✕) and the regression line (—— - ——) are also shown for 10 ovariectomised women.

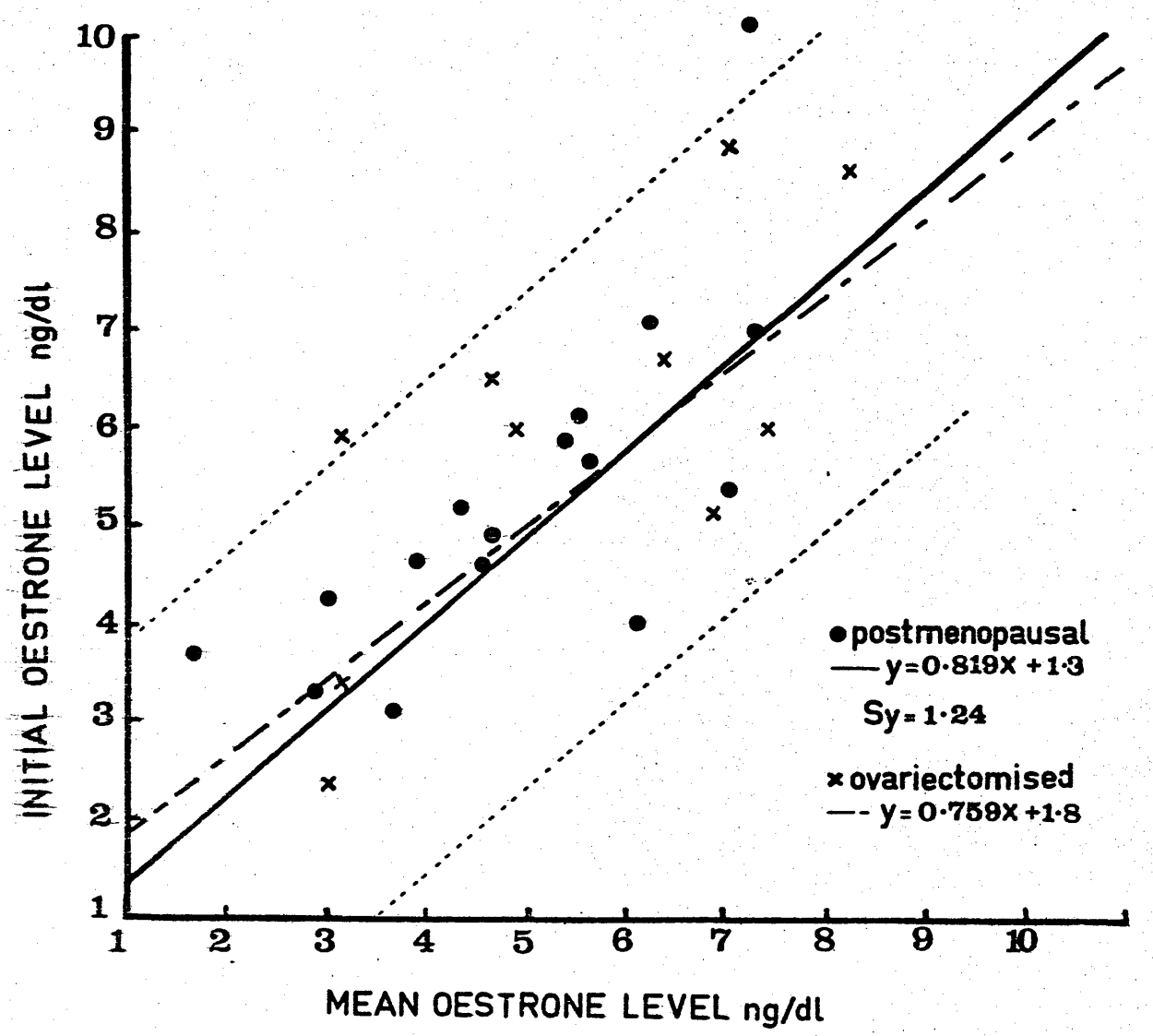

INITIAL OESTRONE LEVEL ng/dl
MEAN OESTRONE LEVEL ng/dl
1
2
3
4
5
6
7
8
9
10
● postmenopausal
—— y=0·819X +1·3
Sy= 1·24
× ovariectomised
— - y= 0·759X +1·8

Fig. 2.6. Correlation of plasma oestradiol concentration in initial blood sample (y axis) and mean value in 9-72 blood samples (x axis) in 16 postmenopausal women (●) to show the possible error of a single oestradiol determination in predicting the mean plasma oestradiol concentration in blood samples obtained every 20-30 minutes for 4-24 hours. The control limits (---) are placed at two standard errors of the estimate S_y from the regression line (——) for the postmenopausal women. The oestradiol values (✕) and the regression line (- — -) are also shown for 10 ovariectomised women.

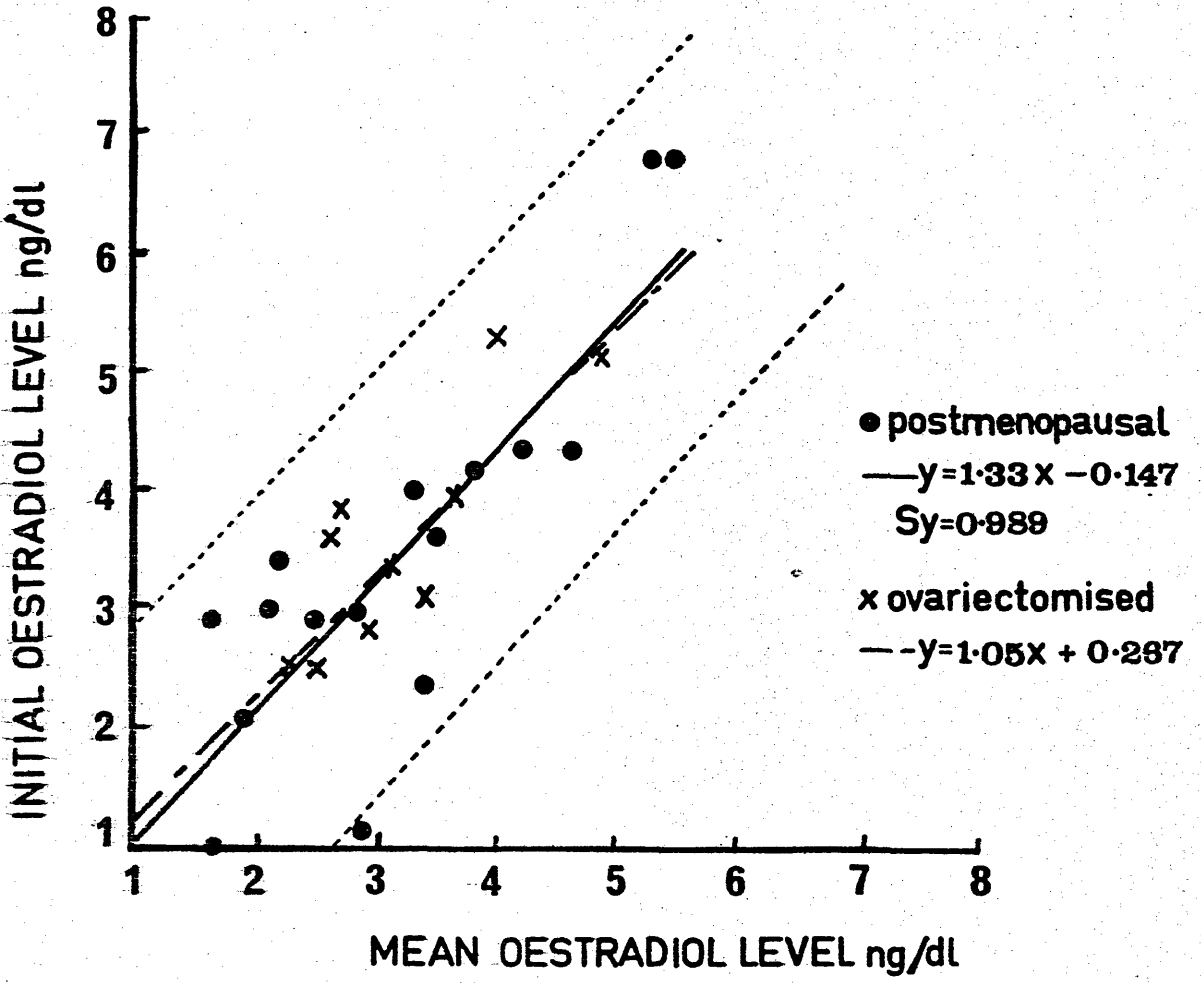

INITIAL OESTRADIOL LEVEL ng/dl
MEAN OESTRADIOL LEVEL ng/dl
1
2
3
4
5
6
7
8
● postmenopausal
—y=1·33x −0·147
Sy=0·989
x ovariectomised
— -y=1·05x + 0·287

Fig. 2.7. Correlation of mean plasma oestrone concentrations in first 9 blood samples (y axis) and mean concentration in 24-72 blood samples (x axis) in 10 postmenopausal women to show the accuracy of the mean level in 9 blood samples obtained every 20-30 minutes for 12-24 hours. The control limits (---) are placed at two standard errors of the estimate S_y from the regression line (——) for the postmenopausal women. The oestrone concentrations ($\bar{x}$) and regression line (- —— -) for 8 ovariectomised women are also shown.

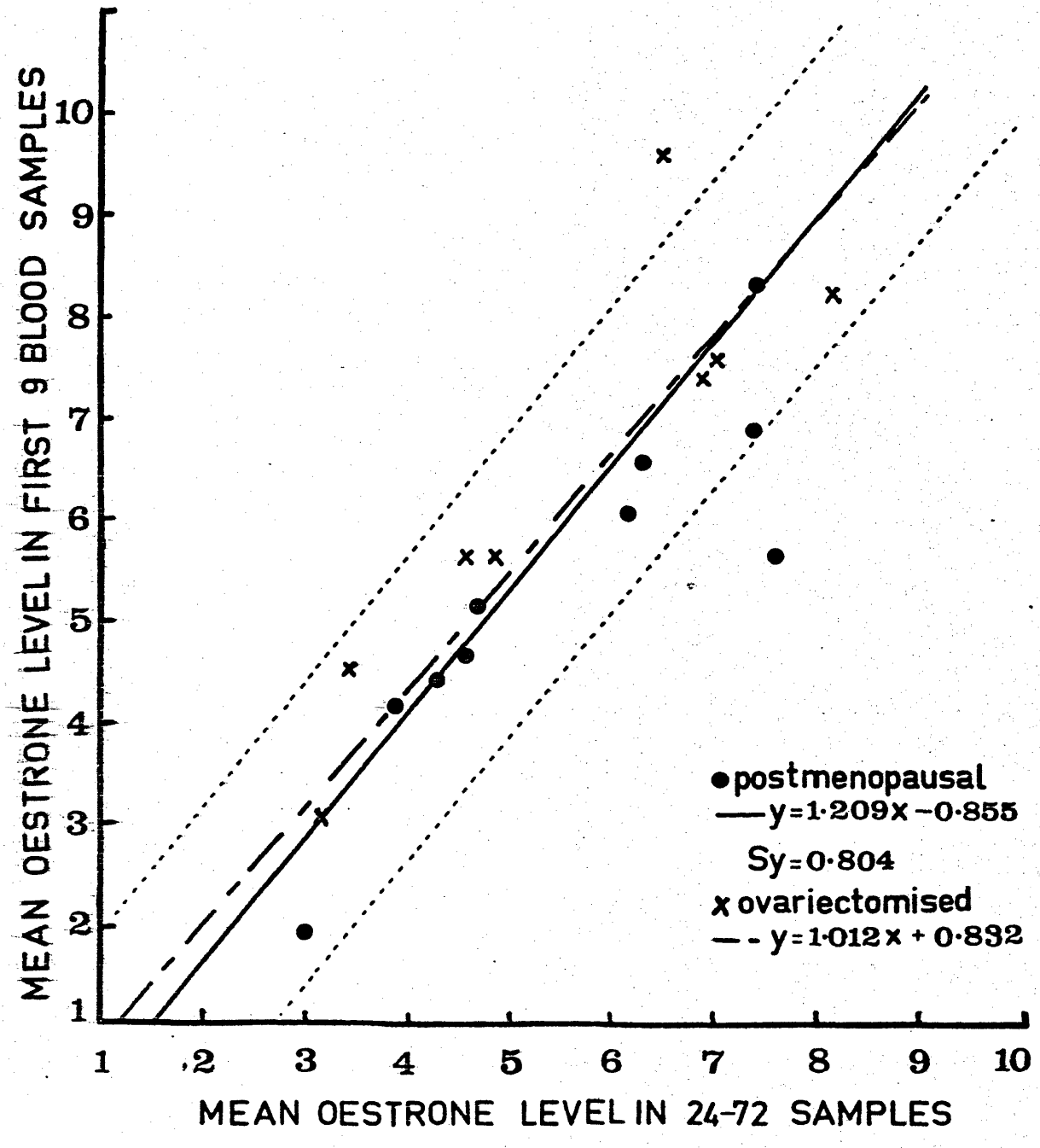

MEAN OESTRONE LEVEL IN FIRST 9 BLOOD SAMPLES
MEAN OESTRONE LEVEL IN 24-72 SAMPLES
1
2
3
4
5
6
7
8
9
10
● postmenopausal
—— y=1·209x −0·855
Sy=0·804
x ovariectomised
— - y=1·012x + 0·832

Fig. 2.8. Correlation of mean plasma oestradiol concentrations in the first 9 blood samples (y axis) and the mean concentrations in 24-72 blood samples (x axis) in 10 postmenopausal women to show the accuracy of the mean oestradiol level determined in blood samples obtained at 20-30 minute intervals for 3-4 hours in predicting the mean level in blood samples obtained every 20-30 minutes for 12-24 hours. The control limits (---) are placed at two standard errors of the estimate S_y from the regression line (——) for the postmenopausal women. The oestrone concentrations (x) and regression line (—— - ——) for 8 ovariectomised women are also shown.

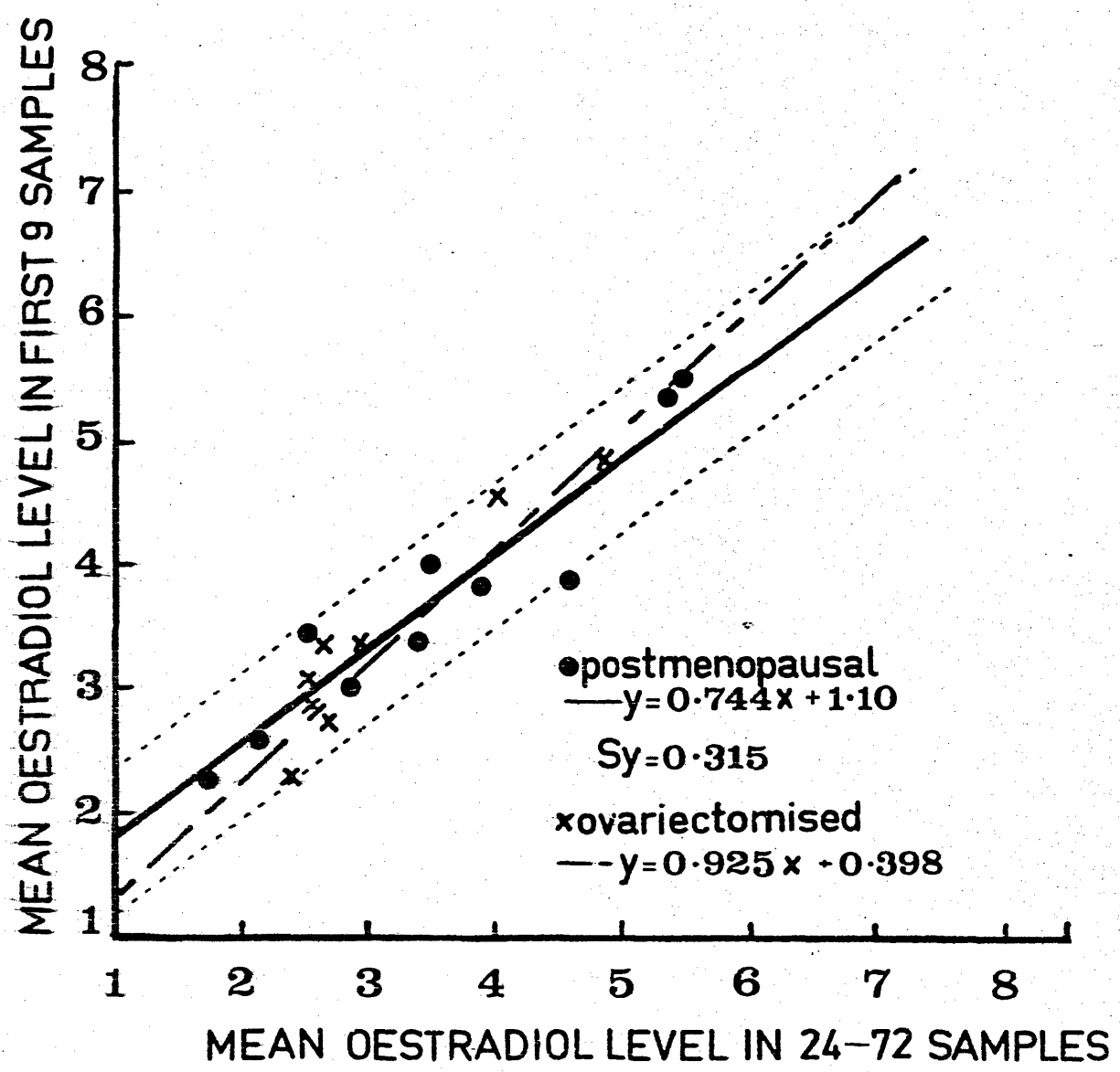
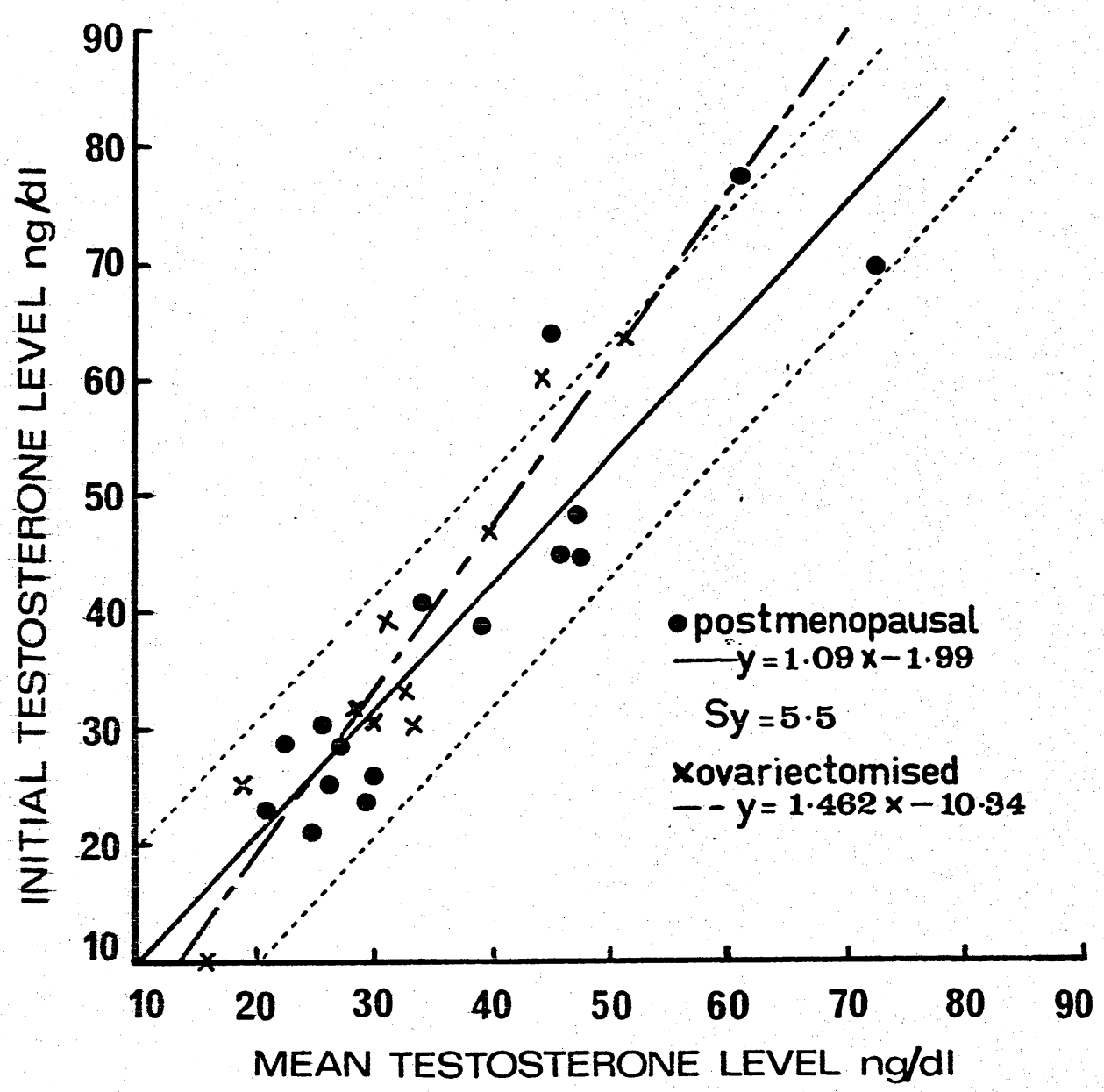

MEAN OESTRADIOL LEVEL IN FIRST 9 SAMPLES
1
2
3
4
5
6
7
8
MEAN OESTRADIOL LEVEL IN 24–72 SAMPLES
●postmenopausal
—y=0·744x +1·10
Sy=0·315
×ovariectomised
– –y=0·925x +0·398

Fig. 2.9. Correlation of plasma testosterone concentration in initial blood sample (y axis) and mean concentration in 9-72 blood samples (x axis) in 16 postmenopausal women to show the possible error of a single plasma testosterone concentration in predicting the mean plasma concentration in blood samples obtained every 20-30 minutes for 4-24 hours. The control limits (---) are placed at two standard errors of the estimate S_y from the regression line (——) for the postmenopausal women. The testosterone concentrations (✕) and regression line (- —— -) are also shown for 10 ovariectomised women.


INITIAL TESTOSTERONE LEVEL ng/dl
MEAN TESTOSTERONE LEVEL ng/dl
10
20
30
40
50
60
70
80
90
● postmenopausal
y = 1·09 x − 1·99
Sy = 5·5
× ovariectomised
– – y = 1·462 x − 10·34

The normal levels and ranges of testosterone in the postmenopausal women were however determined, as for oestrone and oestradiol, from the mean levels during the 4-24 hour sampling periods in 16 postmenopausal women.

The mean concentrations of oestrone and oestradiol, and of testosterone in each of the 16 postmenopausal women are shown in Figures 2.10 and 2.11 respectively. The mean $\pm$ SD concentrations of oestrone, oestradiol and testosterone concentrations in these women were 5.0 $\pm$ 1.7 ng/dl, 3.2 $\pm$ 1.2 ng/dl and 37.1 $\pm$ 15.1 ng/dl respectively. Values of the coefficient of correlation and levels of significance of correlations between oestrone, oestradiol and testosterone concentrations in each of the women, and also with the woman's age, weight and years since the menopause are shown in Table 2.2. The mean oestradiol concentration of each of the 16 postmenopausal women correlated with the woman's weight ($P<0.001$), as shown in Figure 2.12: no other correlations within the 1% level of significance were found.

The ratio of oestradiol to oestrone determined from mean oestradiol and oestrone concentrations was 0.64. The possible error (within 95% confidence limits) in the prediction of the mean oestradiol: oestrone ratio during a sampling period, from the ratio determined from the oestradiol and oestrone concentrations in the first blood sample was, however, 1.4; this error is more than double the actual ratio for women after the menopause and results from independent fluctuations in the plasma oestrone and oestradiol concentrations.

DISCUSSION

Plasma Hormone Concentrations

Oestrogens: The findings in this study have confirmed the conclusions

Fig. 2.10. Mean plasma oestradiol and oestrone concentrations in 16 postmenopausal (P.M.) and 10 ovariectomised (Ov.X.) women.

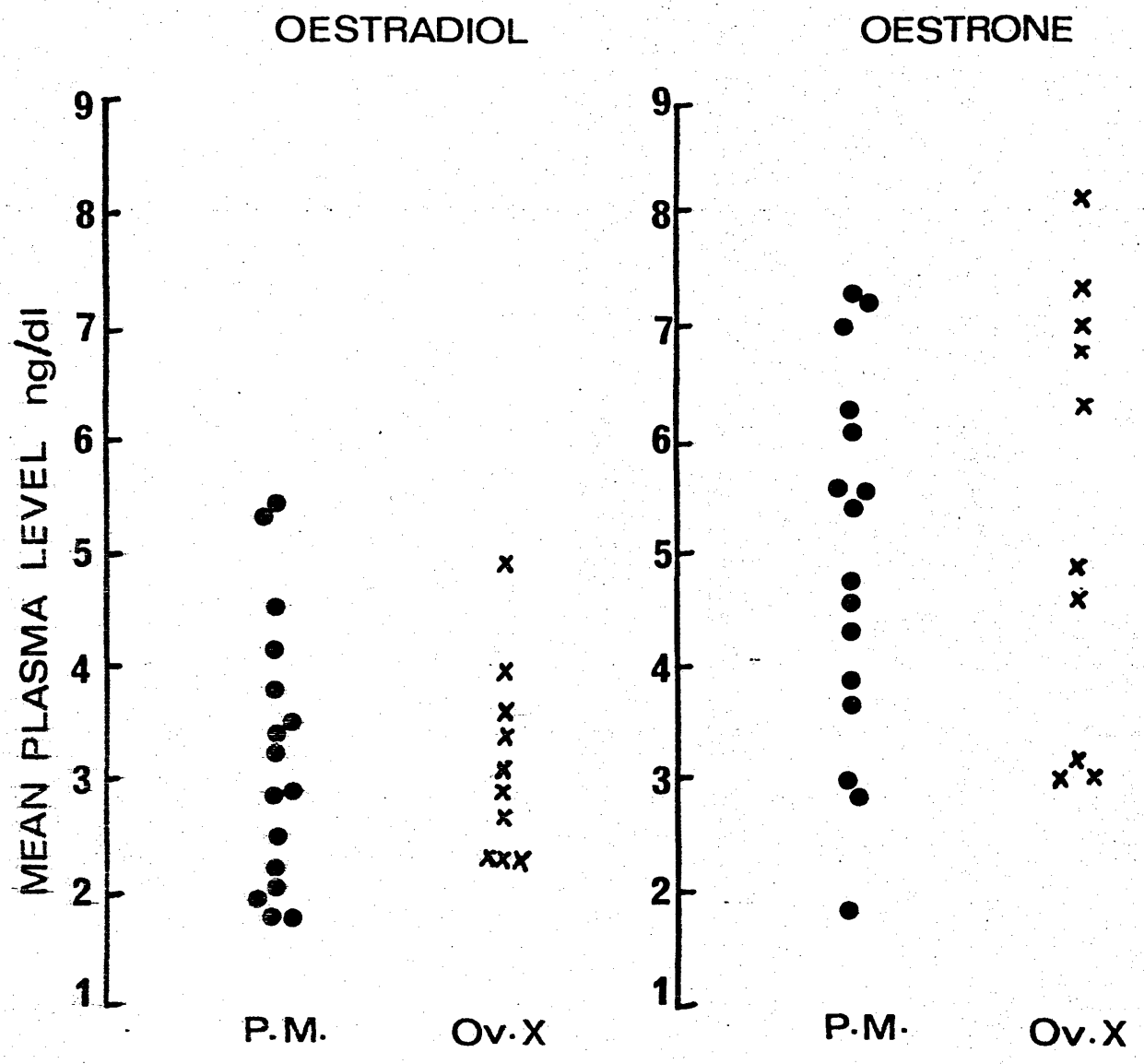

OESTRADIOL
OESTRONE
MEAN PLASMA LEVEL ng/dl
9
8
7
6
5
4
3
2
1
P.M.
Ov.X
P.M.
Ov.X

Fig. 2.11. Mean testosterone concentrations in 16 postmenopausal (P.M.) and 10 ovariectomised (Ov.X.) women.

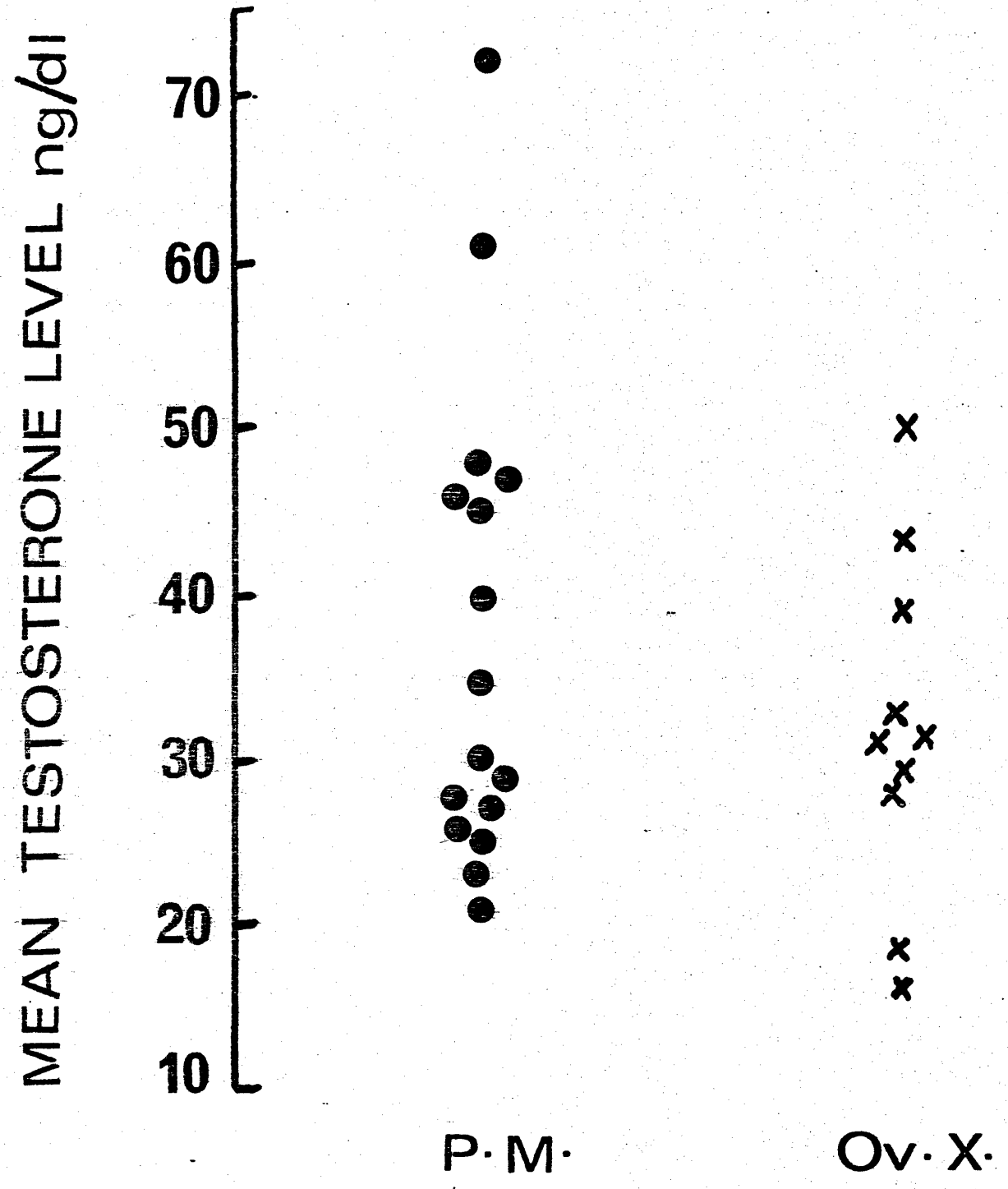
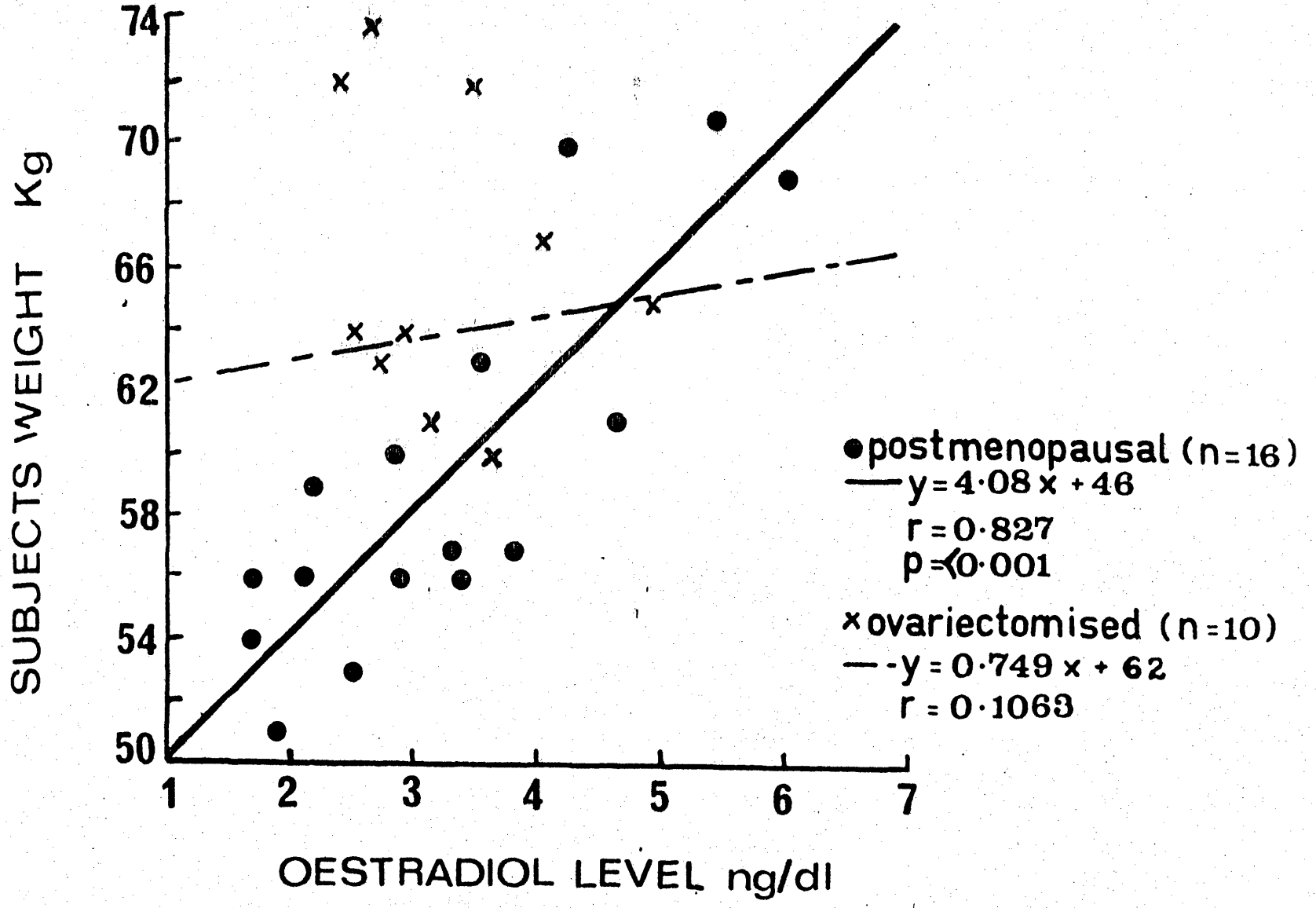

MEAN TESTOSTERONE LEVEL ng/dl
70
60
50
40
30
20
10
P·M·
Ov·X·

Table 2.2. Correlation coefficients between mean plasma oestrone, oestradiol and testosterone concentrations of 16 postmenopausal women (Subject nos. 1-16) and between these hormone concentrations and each woman's age, years since the menopause and weight. Spearman's rank correlation coefficient was calculated for all testosterone correlations as the mean testosterone values were not normally distributed: otherwise the product moment correlation r was determined. * denotes $P < 0.05$; *** denotes $P < 0.001$.

	OESTRONE	OESTRADIOL	TESTOSTERONE
OESTRONE	-	0.323	-0.113
OESTRADIOL	0.323	-	-0.184
TESTOSTERONE	-0.113	-0.184	-
AGE	-0.472	0.239	0.415
YEARS SINCE MENOPAUSE	-0.524*	0.110	0.212
WEIGHT	0.068	0.826***	0.518

Fig. 2.12. Correlations of body weight and mean plasma oestradiol concentration in 16 normal postmenopausal women and 10 ovariectomised women.


SUBJECTS WEIGHT Kg
74
70
66
62
58
54
50
1
2
3
4
5
6
7
OESTRADIOL LEVEL ng/dl
●postmenopausal (n=16)
—y=4·08x+46
r=0·827
p<0·001
×ovariectomised (n=10)
— -y=0·749x+62
r=0·1063

of other investigators that the major circulating hormone deficiency resulting from primary ovarian failure was of oestradiol, the mean level of which was markedly below that observed in premenopausal women during the mid-follicular phase of the ovulatory cycle. However, the mean oestradiol concentration of 3.2 ng/dl in this study was higher than that reported by many investigators (Table 2.3). Urinary excretion and histological studies by Procope and Adlercreutz (1973) and Forleo (1973) have suggested that some ovarian (probably follicular) secretion of oestrogen may occur in women for up to five years after their menopause. Thus, the comparitively high level in this study may be attributable to continued ovarian follicular activity in a proportion of the women who were all within a few years of their menopause. Ovarian secretion of oestrogens in women after the menopause is further discussed in Part 2 b. Alternatively, the high levels may have been due to methodological factors such as non-specific interference in the assay that only became important in this study because the plasma oestradiol concentrations in the postmenopausal women were near the detection limits of the assay. Subsequent to this study, the slope of the standard curve has been further steepened by halving the mass of isotopically labelled oestradiol and using oestradiol of higher specific activity. However, the plasma oestradiol concentrations in postmenopausal women have still been found to be similar to those determined in this study; further studies of the assay technique employed in this study have only confirmed the reliability criteria described in Part 1 (James and Fern, 1978).

Oestradiol levels of about 3 ng/dl have been reported for normal postmenopausal women by Dufau et al. (1970), England et al.

Table 2.3. Mean ± SD plasma oestradiol and oestrone concentrations reported for normal postmenopausal women.

NO.	AGE RANGE IN YEARS	OESTRADIOL ng/dl	OESTRONE ng/dl	AUTHORS AND REFERENCE
6	70-89	1.3 ± 0.5	7.1 ± 6.6	Baird & Guevara, 1969
7	-	1.5 ± 0.8	-	Korenman et al., 1969
15	48-60	-	4.0 ± 2.3	Tulchinsky & Korenman, 1970
7	-	2.5 ± 1.1	-	Dufau et al., 1970
14	70-89	0.7 ± 0.3	2.5 ± 1.3	Longcope, 1971
13	70-90	0.5 ± 0.4	2.2 ± 1.4	Nagai & Longcope, 1971
9	58-81	1.5 ± 0.7	5.1 ± 0.9	Saez et al., 1972
25	50-82	1.3 ± 0.5	3.8 ± 1.4	Rader et al., 1973
-	-	3.3	-	England et al., 1974
4	57-69	1.5 ± 0.7	3.3 ± 1.0	Judd et al., 1974
-	-	1.3 ± 0.1	6.0 ± 1.0	Abraham and Maroulis, 1975
6	49-75	1.1 ± 0.5	2.6 ± 0.5	Judd et al., 1976
10	50-73	2.0 ± 0.6	3.6 ± 1.0	Maroulis and Abraham, 1976
9	41-67	3.4 ± 2.5	4.2 ± 2.6	Yen et al., 1976
20	51-65	2.0 ± 0.5	4.9 ± 2.2	Vermeulen, 1976
-	-	1.4 ± 0.5	3.2 ± 0.9	Somerville et al., 1976
11	52.3 (m)	3.7	4.5	Campbell et al., 1976
60	49-91	1.5	1.5	Chakravarti et al., 1976
18	48-58	1.0 ± 0.4	-	Reyes et al., 1977
18	42-69	-	3.9 ± 1.0	Marshall et al., 1977
11	65 ± 8 (m)	1.1 ± 0.9	3.2 ± 1.8	Borkowski et al., 1977
16	48-62	3.2 ± 1.2	5.0 ± 1.7	Present study

(1974), Yen et al. (1975) and Campbell et al. (1976): the ages of the women in the two former studies were not specified but all the postmenopausal women studied by Yen et al. and Campbell et al. were, like the women in this study, within 10 years of the menopause. However Vermeulen (1976) and Chakravarti et al. (1976) found that the mean plasma oestradiol levels were 2.0 ng/dl and 1.4 ng/dl respectively in women of similar age to those in this study. The low levels of <1.5 ng/dl reported in some studies (Table 2.3) may have resulted from methodological techniques, such as the subtraction of plasma blank values and/or the neglect of significant losses during extraction, that are different from those employed in this study.

If oestradiol circulating in postmenopausal women was derived almost exclusively by the peripheral conversion of oestrone, then, using the mean plasma oestrone concentration of 5 ng/dl found in this study, the transfer constant of 0.06% for the conversion of oestrone to oestradiol reported (in premenopausal women) by Longcope et al. (1968) and the metabolic clearance rates of 1610 L/day and 910 L/day for oestrone and oestradiol respectively (as determined in postmenopausal women by Longcope (1971)), the theoretical plasma oestradiol concentration would be 0.9 ng/dl. This calculated value is below the sensitivity of all assays currently in use. By employing the previously described method of calculation and additional data of Ruder et al. (1972), Olivo et al. (1973) and Anderson et al. (1978), the peripheral conversion of testosterone and of oestrone sulphate can theoretically contribute 0.2 ng/dl and 0.6 ng/dl respectively to the plasma oestradiol concentration. However, further investigations to determine the exact plasma

oestradiol concentrations in normal postmenopausal women are required because of the discrepancies in the reported oestradiol levels, and the possible inaccuracies in calculating the theoretical level from the results of isotopic studies. However, provided allowance has been made for all possible methodological errors, comparisons of values within a subject or subjects are valid, and indeed form the basis of the major conclusions of this study.

In nearly all the studies, the standard deviation of the mean plasma oestradiol concentration of groups of subjects has been large suggesting that the range of levels in postmenopausal women has been wide. The possibility that the weight of the women and/or the sampling procedure employed may account for this wide range of plasma oestradiol levels is discussed below.

This study has confirmed the findings in most other studies (Table 2.3) that oestrone, and not oestradiol, was quantitatively the dominant unconjugated oestrogen circulating in postmenopausal women. The theoretical value for the amount of plasma oestrone derived from androstenedione (which Grodin et al. (1973) suggested was quantitatively the predominant method of oestrone production after the menopause) would be 5.0 ng/dl: this calculation employs data of Longcope et al. (1968, 1969) and Siiteri and MacDonald (1973). The mean plasma oestrone level of 5 ng/dl in this study was similar to the above theoretical value and to that reported by most other investigators (Table 2.3). This mean plasma oestrone value was also similar to that seen during the early to mid-follicular phase of the ovulatory cycle in premenopausal women and therefore, the deficiency of circulating oestrone that resulted from primary ovarian failure was small in comparison with the

deficiency of oestradiol that occurs after the menopause.

One concept of 'hormone replacement therapy' ('HRT') is the prescription of oestrogen to women who have ovarian failure, with the implied intention of repairing biochemically the major hormonal deficiencies that have occurred. The results of this study have shown that the accurate fulfilment of the above concept requires the prescribed oestrogen to increase the plasma oestradiol concentration much more than the plasma oestrone concentration. However, studies described in Part 3 have shown that this biochemical concept cannot be precisely fulfilled by the oral administration of any of the oestrogen preparations currently prescribed as 'HRT'.

Judd et al. (1976) concluded that there was no significant correlation of the plasma oestradiol or oestrone concentrations with the weight of nine normal postmenopausal women. However, if three women prescribed conjugated equine oestrogen therapy are excluded from the analysis (because oestrone sulphate in the mixture may, after absorption, affect the plasma oestradiol concentration), then recalculation of their data gives a significant correlation ($P < 0.01$) of the plasma oestradiol, but not oestrone, concentration with the weight of the women. Although all the postmenopausal women in the present study weighed between 50 and 70 Kg and were within 12% of their ideal weight, the correlation of each woman's mean plasma oestradiol concentration with body weight was highly significant ($P < 0.001$). Different weights of women may therefore have accounted in part for the varying levels and ranges reported for the plasma oestradiol, but not oestrone, concentrations in postmenopausal women (Table 2.3). However, the relationship of plasma oestradiol concentrations to body weight, or more especially

to fat mass (in view of the aromatising potential of adipose tissue (Schindler et al., 1972; Bolt and Gobel, 1972; Nimrod and Ryan, 1975)) requires further study because there was no correlation of the oestradiol concentration with the weight of either ovariectomised women (Part 2 b) or postmenopausal women who were not all of ideal weight (Vermeulen, 1978).

In postmenopausal women the degree of conversion of androstenedione to oestrone (and hence the production rate of oestrone) has been shown to increase with body weight (MacDonald et al., in press). This finding has been supported by the correlation of the plasma oestrone concentration and the body weight of postmenopausal women reported by Vermeulen (1978). The failure to observe a similar correlation in the present study, and in that of Judd et al. (1976), may have occurred because all the women were about normal weight whereas in the studies by Vermeulen, and MacDonald, a proportion of the women were grossly obese and weighed more than 100 Kg.

No correlation of the plasma oestrone concentration with the woman's age was found in this study. In a study by Hemsell et al. (1974), the degree of conversion of androstenedione to oestrone in premenopausal women was however, much lower than occurred in older women. Also in their study, when all women aged between 20-75 years were included in the analysis, a significant correlation between the transfer constant and age was obtained.

The means and ranges of the plasma concentrations of oestrone and oestradiol in this study were, incidentally, similar to those that have been reported in men (Baird and Guevara, 1969; Korenman et al., 1969; Dufau et al., 1970; Nagai and Longcope, 1971; Saez

et al., 1972; England et al., 1974; Hawkins and Oakey, 1974). Although oestrogens in men and postmenopausal women are produced predominantly by the peripheral conversion of androgens, more detailed comparisons may be inaccurate because of the difference in the production and metabolism of androstenedione and, more importantly, of testosterone (Longcope et al., 1969).

Androgens: The mean plasma testosterone concentration of 37 ng/dl in the postmenopausal women in this study was similar to concentrations reported in other studies of postmenopausal women and also of studies in premenopausal women during the mid-follicular phase of the ovulatory cycle (Lobotsky et al., 1964; Judd et al., 1974; Valette et al., 1975; Abraham and Maroulis, 1975; Vermeulen, 1976; Greenblatt et al., 1976; Campbell et al., 1976; Chakravarti et al., 1976; James et al., 1976). These data have therefore provided no evidence of a testosterone deficiency resulting from ovarian failure and thus no physiological basis for the prescription of testosterone as 'hormone replacement therapy' to postmenopausal women.

Plasma androstenedione concentrations in the normal postmenopausal women studied by Grodin et al.(1973); Vermeulen (1976); Greenblatt et al. (1976) and Marshall et al. (1976, 1977) ranged between 93 and 109 ng/dl whereas the concentrations reported by Abraham and Maroulis (1975); Maroulis and Abraham (1976) and Chakravarti et al. (1976) were only 51, 30 and 42 ng/dl respectively. In all these studies, the concentrations were determined in blood samples obtained between 0800 and 1400 hours. Androstenedione and cortisol concentrations fluctuate with a diurnal rhythm (as shown in Figures 2.1-2.4) and because their respective mean levels of

86 ± 27 (SD) ng/dl and 9.0 ± 3.0 (SD) µg/dl were determined in this study in blood samples obtained over a 24 hour period from only four women, a comparison with the levels in the other studies cannot be made. The levels in this study were, however, comparable to concentrations determined in this laboratory in blood samples obtained from premenopausal women (Tunbridge et al., 1973). Thus it seems that the adrenal cortex is the predominant source of androstenedione.

Gonadotrophins: Compared with concentrations in premenopausal women during the mid-follicular phase of the ovulatory cycle, the plasma FSH and LH concentrations in the women in this study showed the 5-10 fold increase that is diagnostic of primary ovarian failure (Odell et al., 1967; Schalch et al., 1968; Jacobs and Murray, 1976; Chakravarti et al., 1976). The increase results from increased pituitary secretion of FSH and LH because the clearance of these glycopeptides is similar in pre- and postmenopausal women (Kohler et al., 1968; Coble et al., 1969). The mean plasma concentration of gonadotrophins was, however, determined in only four of the 16 women in this study and therefore correlations of the gonadotrophin concentrations with each woman's mean plasma oestrogen or androgen concentration were not investigated.

Hormone Patterns and Interrelationships

Cortisol and Androstenedione: In this study the patterns of the plasma concentrations of only cortisol and androstenedione were similar: both showed a diurnal rhythm with other peaks occurring at the same times of the day. The peak in the androstenedione

and cortisol levels at 1300h in Subject no. 2 (Figure 2.2) was associated with an episode of vomiting. The cortisol levels and patterns were otherwise similar to those in the sleep studies of Hellman et al. (1970); Weitzman et al. (1971); de Lacerda et al. (1973) and Czeiler et al. (1976) thus suggesting that the sampling procedure did not usually stress the subjects in this study. The intensive hormone profiles also show that the early morning surge in the plasma levels of cortisol and androstenedione between 0400h and 0800h was composed of a series of peaks. Thus, the conclusions of a study in younger subjects (Tunbridge et al., 1973) that the adrenal secretion of androstenedione and cortisol was regulated predominantly by ACTH and, furthermore, occurred episodically (presumably because ACTH secretion itself occurred episodically - Kreiger and Allen, 1975), are also applicable to postmenopausal women. The calculation of accurate daily blood production rates of cortisol and androstenedione in postmenopausal women therefore demands the determination of accurate mean daily plasma concentrations which, as this study has shown, requires blood samples to be obtained at regular intervals during the day.

Although most of the fluctuations in the plasma cortisol and androstenedione concentrations occurred in parallel, there were occasionally isolated peaks either of cortisol, or more commonly of androstenedione. These changes may have resulted from differences in the metabolic clearance rates, especially as in a later study of an ovariectomised and ACTH-suppressed woman (Part 2 d), marked fluctuations in the plasma androstenedione, but not cortisol, concentrations occurred during a constant infusion of these steroids. The occasional fluctuations of

cortisol and androstenedione levels that were asynchronous were probably not due to ovarian stromal secretion because the phenomenon also occasionally occurred in ovariectomised women (Part 2 b). Theoretically, different biochemical types of ACTH, different responses of the cells of the zona fasciculata and zona reticularis to ACTH and/or episodic production from other plasma androgens could be possible causes also of this phenomenon which requires clarification by further study.

Testosterone: The plasma concentrations of testosterone in the hormone profiles were relatively constant (compared with androstenedione and oestrone) throughout the day, and most of the fluctuations were within the error of the assay method. In this study, the patterns of cortisol and testosterone concentrations were quite unlike and thus ACTH does not appear to regulate testosterone production acutely. Baird et al. (1969) showed by adrenal vein catheterisation studies, that there was a small but significant direct secretion by the adrenal cortex of testosterone. Isotopic studies have suggested that the peripheral conversion of plasma androstenedione may be a major source of plasma testosterone in women (Horton and Tait, 1966; Longcope et al., 1969; Olivo et al., 1973). In this study, the patterns of androstenedione and testosterone concentrations were dissimilar, and the possible mechanisms regulating the peripheral production of testosterone are therefore unclear.

The in vitro studies by Savard et al. (1965), Rice and Savard (1966); Plotz et al. (1967); Mattingly and Huang (1969) and Berman et al. (1973) have all shown that human ovarian stromal tissue can

synthesise androstenedione and testosterone from acetate. Savard et al. (1965) have also suggested that HCG may regulate this production. Greenblatt et al. (1976) and Vermeulen (1976) have shown in vivo that HCG administration increases testosterone production by the postmenopausal ovary. The results of this study showed, however, that any fluctuations in the plasma testosterone concentration were not associated with fluctuations in the plasma levels of LH or, incidentally, FSH. Therefore, gonadotrophins probably do not acutely regulate the production of plasma androgens by the stroma of the postmenopausal ovary. There was no correlation of the mean plasma androgen and gonadotrophin concentrations in the four women studied for 24 hours but the possibility that gonadotrophins may regulate androgen secretion by the postmenopausal ovary in a more gradual manner obviously requires further investigation. Androgen secretion by the postmenopausal is discussed further in Part 2 b.

Oestrone: The plasma oestrone concentrations in most of the profiles in this study showed marked fluctuations which were similar to those occurring in men (Leymarie et al., 1974). Because of the fluctuations, a single blood determination may, as shown in Figure 2.5, be an inaccurate estimate of the plasma oestrone concentration. However the estimation of the oestrone status of women after the menopause, by determining the mean concentration in about 10 blood samples was reasonably accurate for levels > 3 ng/dl; mean levels < 3 ng/dl were, however, often quite inaccurate. The cause of this inaccuracy is unknown but may have been due to slight and variable interference in the

assay by non-specific factors that only significantly affected values near to the limits of sensitivity of the method. Because of the good reliability criteria of the oestrone assay (Part 1) only slight fluctuations in the plasma oestrone concentrations could be attributed to methodological variations. Therefore the marked fluctuations in the plasma oestrone levels were, largely, real.

Others have all shown that the peripheral aromatisation of androstenedione is the major source of plasma oestrone in postmenopausal women. The observation by Vermeulen (1976) of a diurnal rhythm in both oestrone and androstenedione levels determined in blood samples obtained at four hourly intervals from postmenopausal women, suggested that the peripheral conversion of androstenedione was simple. However, Campbell et al. (1976) who employed a two-hourly sampling schedule, found that the morning peak in oestrone concentrations occurred two hours after the androstenedione peak. Furthermore, in some patients, the oestrone levels showed random fluctuations and a diurnal rhythm could not be discerned. By employing an intensive sampling schedule, the present study has shown that the fluctuations in the plasma oestrone concentrations do not have a diurnal rhythm and there is no temporal association with fluctuations in plasma androstenedione concentrations. Adipose tissue has been suggested as the major site of production of plasma oestrone from androstenedione (Siiteri and MacDonald, 1973; Nimrod and Ryan, 1975), although other tissues such as the liver (Smuk and Schwers, 1977) and fibroblasts (Schweinkert et al., 1975) have the necessary aromatising enzymes Adipose tissue has also been suggested as an extravascular store

of oestrogen which releases oestrone and oestradiol into the circulation (Twombley et al. 1967; Bleau et al., 1974). Isotopic studies by Longcope (1972 a & b) have also demonstrated that plasma oestrone may be formed by deconjugation of plasma oestrone sulphate and by the oxidation of plasma oestradiol, and Olivo et al. (1973) have detected conversion of plasma testosterone to oestrone. However, the profiles in this study have shown that there is no temporal association between fluctuations in plasma oestrone and those in plasma oestradiol or testosterone concentrations. Although episodic production from peripheral sources such as oestradiol, testosterone and oestrone sulphate, and/or episodic release from aromatising tissues or extravascular 'stores' such as adipose tissue may have accounted for the fluctuations in the plasma oestrone levels observed in this study, the mechanisms regulating any such production remain unclear.

The fluctuations in the plasma oestrone concentrations may, however, also have resulted in part from changes in the rate of metabolic clearance of oestrone. Oestrone is largely cleared by the liver whose blood flow may fluctuate markedly after ingestion of food (Brauer, 1963). In this study, food was not restricted and the effects of food ingestion were not investigated. Although posture was not kept constant throughout the sampling period, the isotopic studies of Flood et al. (1973) have shown that alterations in posture do not affect the plasma oestrone level, but only the metabolic clearance and production rates of oestrone. It is perhaps of interest that in post - menopausal women with breast cancer, equilibrium during isotopic

infusions of oestrone has been difficult to attain (Hembree et al., 1969; Pearlman et al., 1969) thus suggesting that alterations in the metabolic clearance rate occurred during the infusions. However, Longcope and Tait (1971) found in younger subjects that the concentrations of radioactivity as free oestrone and as the product free oestradiol, remained relatively constant throughout infusions of ^{3}H-oestrone that lasted up to 12 hours. These results suggested that, in their subjects at least, the metabolic clearance did not alter during the infusions. From consideration of Longcope's data, fluctuations in the plasma levels of oestrone would not, therefore be expected.

In summary, therefore, it is presently unclear whether the marked and apparently random fluctuations in the plasma concentrations may have been due to episodic production or release of oestrone or to fluctuations in the metabolic clearance rate of oestrone. The fluctuations must, however, have cast some doubt upon the validity of conclusions based on plasma oestrone concentrations determined in single, or only a few, plasma samples. Such studies have included those made by Siiteri and MacDonald (1973) suggesting that oestrone production is abnormal in postmenopausal women with endometrial carcinoma, and by Marshall et al.(1977) suggesting that osteoporosis in postmenopausal women is related to a deficiency of circulating oestrone.

Oestradiol: The fluctuations in the plasma oestradiol concentrations throughout the sampling period in the women in this study were not usually as marked as those described above for oestrone, and therefore the possible errors in normal postmenopausal women in

the prediction of the mean oestradiol concentration from the concentration in a single blood sample and from the level in nine blood samples were slightly lower than those for oestrone. This study did show however that a single blood determination may sometimes be an inaccurate estimate of the oestradiol status of postmenopausal women. However, an estimate of the oestradiol status from the mean concentration in about 10 blood samples was reasonably accurate if the concentrations exceeded 2 ng/dl. However the error exceeded 30% when mean levels were $<$ 2 ng/dl. The cause of this inaccuracy is unknown, but as in the case of oestrone, may have been due to more marked variations in the production or metabolism of oestradiol at low levels, or perhaps to methodological problems as previously discussed for oestrone.

Fluctuations of up to 25% of the oestradiol concentration may have been attributable to methodological variations. The plasma levels were necessarily not determined in duplicate in the assay. The determination of each plasma oestradiol concentration in duplicate would however alter the value by less than 10% on average, and not more than 20% - (these percentages are the mean and highest coefficient of variation respectively for the male pool, and also of more than 40 blood samples assayed in duplicate in a single assay and whose values ranged from 1.5-5.0 ng/dl (Part 3). Catabolism of oestradiol by red blood cells before separation of the plasma may also have accounted for some methodological variation. In vitro studies (Migeon et al., 1962) and in vivo studies (Longcope et al., 1968) have shown that red blood cells may metabolise plasma oestradiol and oestrone. Minor variations in the sampling and plasmaphoresis technique may

therefore have caused some fluctuations in the plasma oestradiol concentrations in the postmenopausal women because it was not always possible to undertake the plasmaphoresis of the 1000 blood samples in the exact manner outlined in the protocol. However, the theoretical limit of catabolism of oestrogens by red blood cells could not be more than 5%.

In this study, some of the fluctuations in the plasma oestradiol concentration exceeded the changes possibly attributable to methodological variations, and they must therefore be attributed to alterations in the production and/or metabolic clearance rates of oestradiol. These fluctuations were not temporally associated with fluctuations in the plasma concentrations of oestrone, testosterone or androstenedione, which are the unconjugated prehormones from which plasma oestradiol is predominantly produced in the periphery (Longcope et al., 1969; Longcope, 1971). Thus the mechanisms regulating the peripheral production of oestradiol from these prehormones require further study. Theoretically, episodic interconversion of oestradiol with plasma oestrone sulphate may also have caused fluctuations in the plasma concentration, but the exact role of conjugates in the production of plasma oestrogens after the menopause has yet to be defined. Fluctuations in plasma oestradiol may, like those of oestrone, also be caused by episodic release from oestrogen stores and/or sudden alterations in the metabolic clearance rate of oestradiol. Fluctuations in the plasma oestrogen levels were probably not due to episodic secretion by the stroma of the postmenopausal ovary because similar fluctuations occurred in ovariectomised women (Part 2 b).

Gonadotrophins: This study has confirmed the findings of others including Nillius and Wide (1970), Yen and Tsai (1971) and Campbell et al. (1976) that the pattern of the plasma gonadotrophin concentrations in postmenopausal women does not show a diurnal rhythm but random fluctuations. Because the clearance of gonadotrophins in pre- and postmenopausal women is similar (Kohler et al., 1968; Coble et al., 1969), the findings in this study have also supported the conclusions of Yen et al. (1972 a & b) and Root et al. (1972) that gonadotrophin production after the menopause is episodic. The determination of FSH and LH concentrations in postmenopausal women for research purposes therefore requires their determination in several blood samples.

Although several studies have demonstrated that the negative feedback effect of oestrogen at hypothalamic-pituitary level is still operative in women with ovarian failure (Schalch et al., 1968; Odell et al., 1968; Nillius and Wide, 1970; Wallach et al., 1970; Tsai and Yen, 1971; Franchimont et al., 1972; Wise et al., 1973; Yen et al., 1975; Furuhashi et al., 1977), this study has shown that the fluctuations in the gonadotrophin concentrations are not mediated by feed back of oestrone or oestradiol, or of their androgenic prehormones. The lack of association of fluctuations in either of the gonadotrophins with fluctuation in plasma oestrogen or androgen concentrations has also shown that gonadotrophins do not regulate acute secretion of steroids, particularly androgens, in postmenopausal women. This observation is not therefore consistent with the conclusions of some *in vitro* and *in vivo* studies which have suggested that there is significant androgen (but not oestrogen) secretion by the postmenopausal ovary, and which have previously been discussed.

Part 2 b) THE CONTRIBUTION OF THE POSTMENOPAUSAL OVARY TO PLASMA STEROIDS

The objectives for studying the role of the postmenopausal ovary in the production of plasma oestrogens have been described previously. Briefly, some previous studies have demonstrated that the postmenopausal ovary may secrete significant amounts of androgenic prehormones, particularly testosterone, but not oestrogens. Other studies, including those described in Part 2 a, have suggested that ovarian secretion of oestrogens may also occur in some women within a few years of their menopause. All these studies have, however, never allowed for both fluctuations in the hormone levels that occur in postmenopausal women and contributions by the adrenal cortex and periphery, the alternative sources of plasma oestrogens. In the present study, the contribution of the ovary to plasma oestrogens after the menopause was investigated by employing intensive hormone profile techniques to compare postmenopausal with ovariectomised women with respect to the hormone levels, patterns and interrelationships, and the effects of depletion of peripheral sources and suppression of adrenal secretion of steroids. The endocrine function of the ovary was also examined in a longitudinal study of a woman with amenorrhoea and biochemical evidence of primary ovarian failure.

METHOD

The hormone profiles of the four postmenopausal women (Subject Nos. 1-4, described in Part 2 a) in whom plasma steroid and gonadotrophin concentrations were determined in blood samples obtained every 20 minutes for 24 hours were compared with profiles in two ovariectomised women (Subject Nos. 17 & 18) from whom blood samples

were also obtained every 20 minutes for 24 hours. These two ovariectomised women aged 46 and 49 years had been ovariectomised 0.4 and 14 years previously (one for endometriosis and one for fibroids and ovarian fibroma) and were, like the four postmenopausal women, within 12% of their ideal weight for their height (Geigy, 1970).

In addition the mean hormone concentrations in the 16 postmenopausal women (Subject Nos 1-16) described in Part 2 a were compared with mean concentrations in the two ovariectomised women described above, and a further eight ovariectomised women (Subject Nos 19-26) from whom blood samples were obtained every 30 minutes for 4-14 hours. The bilateral ovariectomies in these eight women had been performed at the time of hysterectomy because of carcinoma of the cervix (3 women), endometriosis (2 women), fibroids (1 woman) and abnormal (dysfunctional) uterine bleeding (2 women). Other clinical details of the postmenopausal and ovariectomised women are given in Table 2.4. The mean age of 47.9 $\pm$ 4.1 (SD) years for the ten ovariectomised women was significantly lower ($P < 0.01$) than the mean age of 52.4 $\pm$ 3.7 years for the postmenopausal women. The interval since ovariectomy was not, however, significantly different ($P < 0.05$ Mann Whitney U test) from the duration of amenorrhoea in the postmenopausal women. The mean weight (66.7 $\pm$ 4.9 Kg) of the ovariectomised women was significantly greater ($P < 0.01$) than the mean weight (59.3 $\pm$ 6.1 Kg) of the postmenopausal women. The mean percentage differences of the body weight from the mean ideal weight of medium framed women of the same height in the ten ovariectomised women and the 16 postmenopausal women were 11.3 $\pm$ 8.7% and 5.8 $\pm$ 4.4% respectively. This difference

Table 2.4. Age, years since menopause (MP) or ovariectomy (OV), weight, %difference from ideal weight (% diff.) fat mass (Kg) and mean ± SD oestrone (E_1), oestradiol (E_2) and testosterone (T) concentrations (ng/dl) in blood samples obtained every 20-30 minutes for 4-24 hours from 16 normal postmenopausal women (Nos. 1-16), ten ovariectomised women (Nos. 17-26) and two obese postmenopausal women (Nos. 36 & 39). Mean androstenedione concentrations (A, ng/dl) were determined only in those subjects from whom blood samples were collected for 24 hours.

NO	AGE	YEARS MP/OV	WT (Kg)	% DIFF	FAT MASS	E_1	E_2	T	A
1	54	4.5	56	+ 5.6	3.6	6.1 ± 1.2	2.1 ± 0.9	25.3 ± 4.4	66.0 ± 28.0
2	51	3.0	53	- 2.0	-1.0	4.3 ± 1.2	2.5 ± 0.9	45.0 ± 8.6	94.4 ± 56.2
3	51	4.0	56	+ 2.5	1.7	4.6 ± 2.0	3.4 ± 1.4	26.2 ± 4.1	64.0 ± 21.0
4	55	5.0	69	+12.0	7.5	7.3 ± 2.0	5.5 ± 1.2	47.8 ± 6.5	120.5 ± 34.6
5	55	4.5	54	+ 2.6	2.0	3.0 ± 2.0	1.7 ± 1.6	47.2 ±10.5	65.7 ± 27.3
6	54	1.9	61	+12.0	8.0	6.2 ± 1.5	4.6 ± 0.9	61.8 ± 8.8	- -
7	53	3.0	63	+ 1.0	1.5	4.7 ± 1.0	3.5 ± 0.9	21.0 ± 5.2	- -
8	51	0.4	57	+11.6	8.0	7.3 ± 3.5	3.8 ± 1.4	46.0 ± 1.4	- -
9	48	0.4	56	+ 3.7	2.0	7.0 ± 1.6	1.7 ± 1.0	27.2 ± 3.1	- -
10	62	8.0	70	+11.9	8.5	1.7 ± 0.9	4.2 ± 0.8	72.9 ± 9.1	- -
11	51	2.6	51	- 0.5	-0.5	2.9 ± 0.5	1.9 ± 0.6	34.5 ± 6.1	- -
12	51	0.9	57	+ 5.5	3.0	5.4 ± 0.4	3.3 ± 1.4	29.8 ± 2.3	- -
13	54	3.0	60	+ 3.3	3.0	3.9 ± 1.6	2.8 ± 0.7	22.7 ± 5.1	52.2 ± 30.5
14	51	1.6	59	- 9.0	-1.0	3.7 ± 1.0	2.2 ± 1.0	39.6 ± 5.0	52.2 ± 17.6
15	49	2.1	56	+ 1.0	1.7	5.5 ± 0.6	2.9 ± 1.7	29.7 ± 4.0	- -
16	49	0.9	71	+ 9.1	6.0	5.6 ± 2.6	5.4 ± 1.0	26.2 ± 3.5	- -

cont.

Table 2.4. (cont.)

NO	AGE	YEARS MP/OV	WT (Kg)	% DIFF	FAT MASS	E_1	E_2	T	A
17	46	0.4	56	+ 4.6	3.0	6.4 ± 3.2	4.0 ± 1.4	32.9 ± 6.4	93.8 ± 51.7
18	49	14.0	65	+ 1.5	1.0	4.6 ± 1.0	4.9 ± 1.0	18.4 ± 6.2	48.6 ± 17.9
19	50	3.0	63	+ 2.4	1.5	4.9 ± 1.0	2.7 ± 0.9	50.7 ± 18.5	- -
20	42	4.0	72	+14.1	9.5	3.2 ± 1.1	2.3 ± 0.4	29.6 ± 6.7	- -
21	47	1.1	61	+11.0	7.0	8.2 ± 0.7	3.1 ± 0.9	28.0 ± 4.2	- -
22	46	0.4	64	0.0	0.0	3.1 ± 2.1	2.9 ± 0.6	32.9 ± 5.2	- -
23	54	5.0	72	+24.0	14.0	3.0 ± 1.4	3.0 ± 0.9	31.2 ± 6.7	- -
24	49	6.0	60	+20.0	10.0	7.4 ± 1.1	3.6 ± 1.0	39.7 ± 6.2	- -
25	42	2.0	64	+16.6	10.0	7.0 ± 1.9	2.5 ± 1.1	16.5 ± 8.3	- -
26	53	15.0	74	+19.0	10.0	6.9 ± 1.5	2.6 ± 0.7	43.8 ± 13.4	- -
36	52	1.8	71	+31.0	17.0	4.3 ± 1.0	5.5 ± 0.6	42.9 ± 5.0	85.0 ± 29.3
39	56	2.1	107.5	+73.0	46.0	8.6 ± 1.3	4.5 ± 0.9	33.4 ± 8.1	74.0 ± 32.0

between the two groups was not statistically significant.

A 51 year old woman (Subject No. 8) who had not had periods for four months and who complained of flushes, but not superficial dyspareunia was studied longitudinally for ten months commencing on 16.1.76. During the first four months of the study, piperazine oestrone sulphate was prescribed cyclically but this was discontinued on 28.5.76. The oestrogen therapy was recommenced six months later on 29.11.76 because flushes had then recurred. During the study, periods occurred on 23.9.75, 4.4.76, 6.6.76, 8.8.76 and 1.10.76. Plasma FSH, LH, oestradiol and progesterone concentrations were determined in samples obtained at intervals during the ten month study. The progesterone concentrations were determined using the supraregional assay service.

The effects on plasma steroid concentrations of adrenal suppression and consequent peripheral depletion of the alternative sources of oestrogen production were determined in four postmenopausal women (Subject Nos. 7,28, 30 & 33) and seven ovariectomised women (Subject Nos. 19, 21, 27, 29, 31, 32 & 34) after treatment with dexamethasone in a dose of 1.5 mg daily for seven days. The indications for the ovariectomies in these women were carcinoma of the cervix (4 women), fibroids, adenomyosis and dysfunctional uterine bleeding (one woman each). Other clinical details of the women are summarised in Table 2.5. Plasma androstenedione, cortisol, testosterone oestrone and oestradiol concentrations were determined in blood samples obtained every 30 minutes for 4-12 hours from these 11 women. The mean age of 52.3 $\pm$ 3.0 years of the four postmenopausal women was similar to the age 50.6 $\pm$ 2.9 years of the seven ovariectomised women. The mean interval of amenorrhoea of 2.7 $\pm$ 2.5 years in the

Table 2.5. Clinical details and mean ± SD plasma oestrogen and androgen concentrations (ng/dl) in blood samples obtained every 30 minutes for 4-14 hours from postmenopausal (PM) or ovariectomised (OV) women after treatment with dexamethasone 1.5 mg daily for one week.

NO	AGE	WT (Kg)	% DIFF	YEARS MP/OV	E_1	E_2	A	T	
7	53	63	+ 1.0	3.1	2.9 ± 0.4	1.6 ± 0.6	27.0 ± 1.6	12.8 ± 3.6	
28	49	51	- 6.0	1.0	1.2 ± 0.3	2.6 ± 0.6	27.8 ± 3.0	31.4 ± 8.9	Postmenopausal
30	51	73	+37.0	0.6	2.4 ± 0.6	2.5 ± 0.4	<26.6	28.0 ± 5.3	
33	56	54	+ 8.0	6.0	2.0 ± 0.9	<1.0	"	<10.0	
19	50	63	+ 2.4	3.0	1.0 ± 0.1	1.3 ± 0.5	27.1 ± 3.4	18.2 ± 6.3	
21	47	61	+11.0	1.1	1.6 ± 0.9	1.4 ± 0.4	<26.6	<10.0	
27	50	58.5	+11.6	0.3	1.3 ± 0.4	2.1 ± 0.5	27.6 ± 2.5	10.5 +0.7	
29	55	54	+ 5.4	8.0	1.4 ± 0.8	2.0 ± 0.6	<26.6	<10.0	Ovariectomised
31	50	60	+11.0	15.0	1.3 ± 0.3	1.9 ± 0.4	"	"	
32	54	58	+17.0	12.0	1.0	1.6 ± 0.4	"	"	
34	48	56	+10.0	8.0	2.1 ± 1.0	1.9 ± 0.6	"	"	

postmenopausal women was not significantly different from the interval of 6.8 ± 5.6 years since ovariectomy in the other women. The weights and percentage differences from ideal weights of the postmenopausal and ovariectomised women were also similar, although the range of weights of 51-73 Kg in the postmenopausal women was wider than the range of 54-63 Kg in the ovariectomised women.

The complete clinical and sampling deatils of each of the postmenopausal and ovariectomised women in this study are given in Appendix IV. All the ovariectomised women had been castrated premenopausally.

RESULTS

The hormone concentrations, patterns and interrelationships in the profiles of the two ovariectomised women (Subject Nos. 17 & 18) shown in Figures 2.13 and 2.14 were similar to those of the four postmenopausal women in Table 2.1. There was no significant difference in the mean hormone concentrations between the two groups of women.

The patterns of plasma oestrone, oestradiol and testosterone concentrations in the eight other ovariectomised women were similar to those described above for the two ovariectomised women and those described in Part 2 a for the postmenopausal women. In the ovariectomised women, the plasma oestrone and oestradiol concentrations determined in a single blood sample did not correlate exactly with the mean levels determined in blood samples obtained over a 4-24 hour period as shown in Figures 2.5 and 2.6 respectively. However, the error in predicting the mean 12-24 hour level from the mean level in nine blood samples was, like that in the postmenopausal women, small and acceptable for this study provided mean concentrations

Figures 2.13. Plasma steroid and gonadotrophin concentrations in blood samples obtained every 20 minutes for 24 hours from an ovariectomised woman (Subject No. 17).

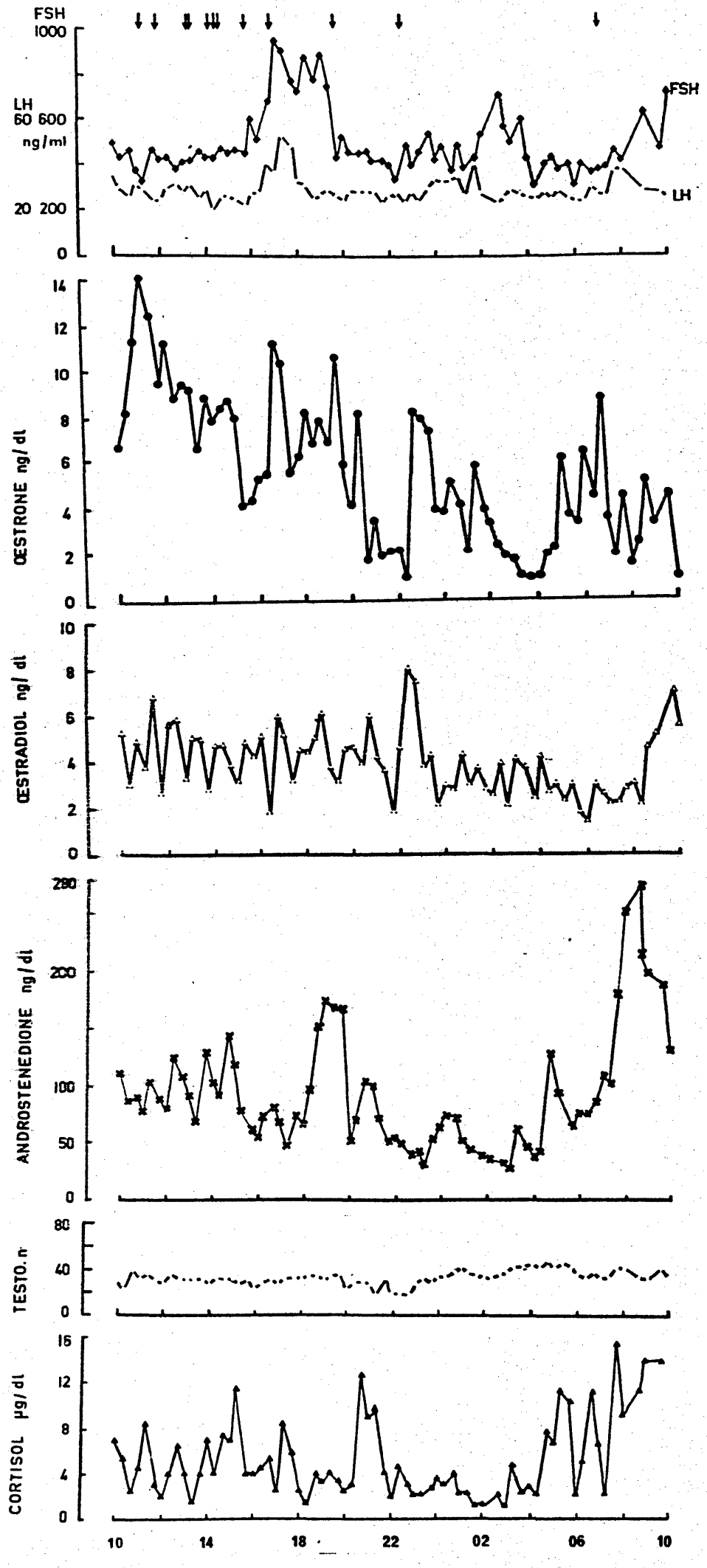
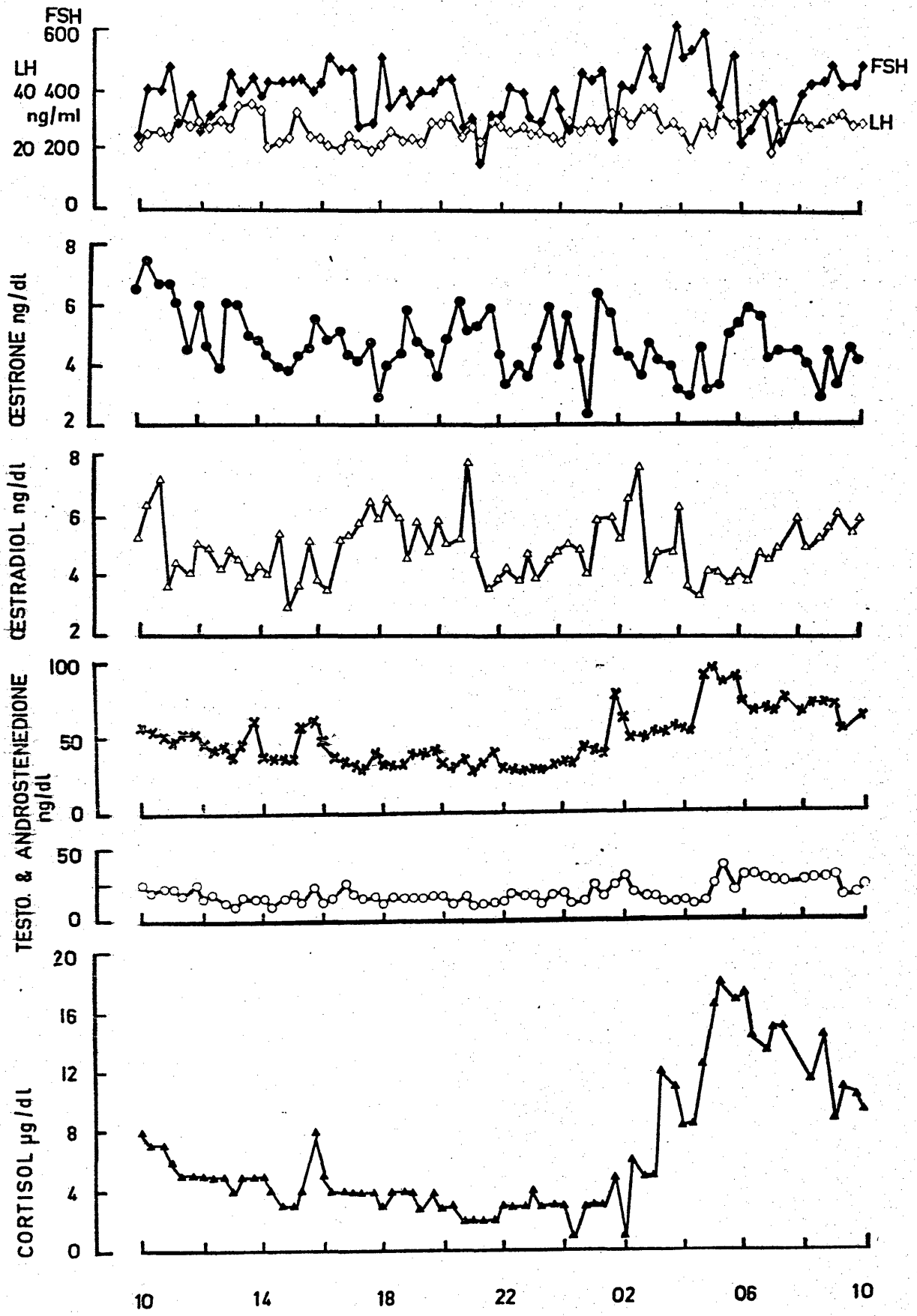

FSH
1000
LH
60 600
ng/ml
20 200
0
FSH
LH
ŒSTRONE ng/dl
14
12
10
8
6
4
2
0
ŒSTRADIOL ng/dl
10
8
6
4
2
0
ANDROSTENEDIONE ng/dl
200
100
50
0
TESTO. n
80
40
0
CORTISOL µg/dl
16
12
8
4
0
10
14
18
22
02
06
10

Figure 2.14. Plasma steroid and gonadotrophin concentrations in blood samples obtained every 20 minutes for 24 hours from an ovariectomised woman (Subject No. 18).


FSH
600
LH
40 400
ng/ml
20 200
0
FSH
LH
ŒSTRONE ng/dl
8
6
4
2
ŒSTRADIOL ng/dl
8
6
4
2
TESTO. & ANDROSTENEDIONE ng/dl
100
50
0
50
0
CORTISOL µg/dl
20
16
12
8
4
0
10
14
18
22
02
06
10

of oestrone and oestradiol were above 3 ng/dl and 2 ng/dl respectively. The possible error in predicting the mean testosterone concentration from a single blood sample obtained from ovariectomised women was 7.5 ng/dl whereas the error in the postmenopausal women was 11.0 ng/dl.

For the purpose of comparing plasma concentrations between postmenopausal and ovariectomised women, the testosterone, oestradiol and oestrone values in each woman in this study were the mean concentrations in 9-72 blood samples obtained over 4-24 hour periods. The mean concentrations of oestrone and oestradiol, and of testosterone in each of the ovariectomised women are shown with those of the post - menopausal women in Figures 2.10 and 2.11 respectively, and in Table 2.4.

The mean concentrations of oestrone, oestradiol and testosterone in the group of ovariectomised women were 5.5 $\pm$ 2.0 ng/dl, 3.2 $\pm$ 1.8 ng/dl and 32.4 $\pm$ 10.5 ng/dl respectively and in the group of post - menopausal women were 5.0 $\pm$ 1.7 ng/dl, 3.2 $\pm$ 1.2 ng/dl and 37.1 $\pm$ 15.1 ng/dl respectively. There were no significant differences in the mean concentrations of oestrone, oestradiol and testosterone between the groups of ovariectomised and postmenopausal women.

In the 10 ovariectomised women, there was no significant correlation between the mean oestrone, oestradiol and testosterone concentrations or between these concentrations and the age, weight or percentage difference from the ideal weight of the women. However, in the five ovariectomised women who were within 12% of their ideal body weight, there was a significant correlation ($P < 0.01$) of the plasma oestrone (but not oestradiol) concentration and the percentage difference from ideal weight (but not the actual body weight). In the normal postmenopausal women (Table 2.4), all the

correlations were insignificant except for two highly significant correlations ($P < 0.001$) of the mean plasma oestradiol concentration and the body weight, and the percentage difference from the ideal body weight.

The profiles of the four postmenopausal women and seven ovariectomised women treated with dexamethasone 1.5 mg daily for one week are shown in Figures 2.24, 2.23,2.25 for Subject Nos. 7, 19, 21, 27-34 respectively. The plasma concentration of cortisol was always undetecable. The plasma androgen and oestrogen concentrations in most women were at or near the limits of detection of the assays, although occasionally there were small independent peaks in the hormone levels which were usually within the methodological error of the assays. Values of oestrone, oestradiol testosterone and androstenedione which were at or below the limits of sensitivity of the method have been designated 1 ng/dl, 1 ng/dl, 10 ng/dl and 26.6 ng/dl for the purpose of the calculations in this study. The mean oestrone, oestradiol, testosterone and androstenedione concentrations in each woman after the dexamethasone treatment (but before the commencement of any infusions) are shown in Table 2.5. Using the Mann Whitney U test, there were no significant differences in the mean oestrogen or androgen concentrations (after dexamethasone) between the postmenopausal and the ovariectomised women. In the four postmenopausal women treated with dexamethasone, there were negative correlations ($P < 0.01$) of the mean plasma oestradiol concentration and their age (Figure 2.15) and years since the menopause (Figure 2.16). The negative correlations of the plasma testosterone concentrations and the age of these same postmenopausal women and the years since their menopause (Figure 2.17) were almost

Figure 2.15. Correlation of mean plasma oestradiol concentration and age in four postmenopausal women (●) and seven ovariectomised women (×) after dexamethasone treatment for one week.

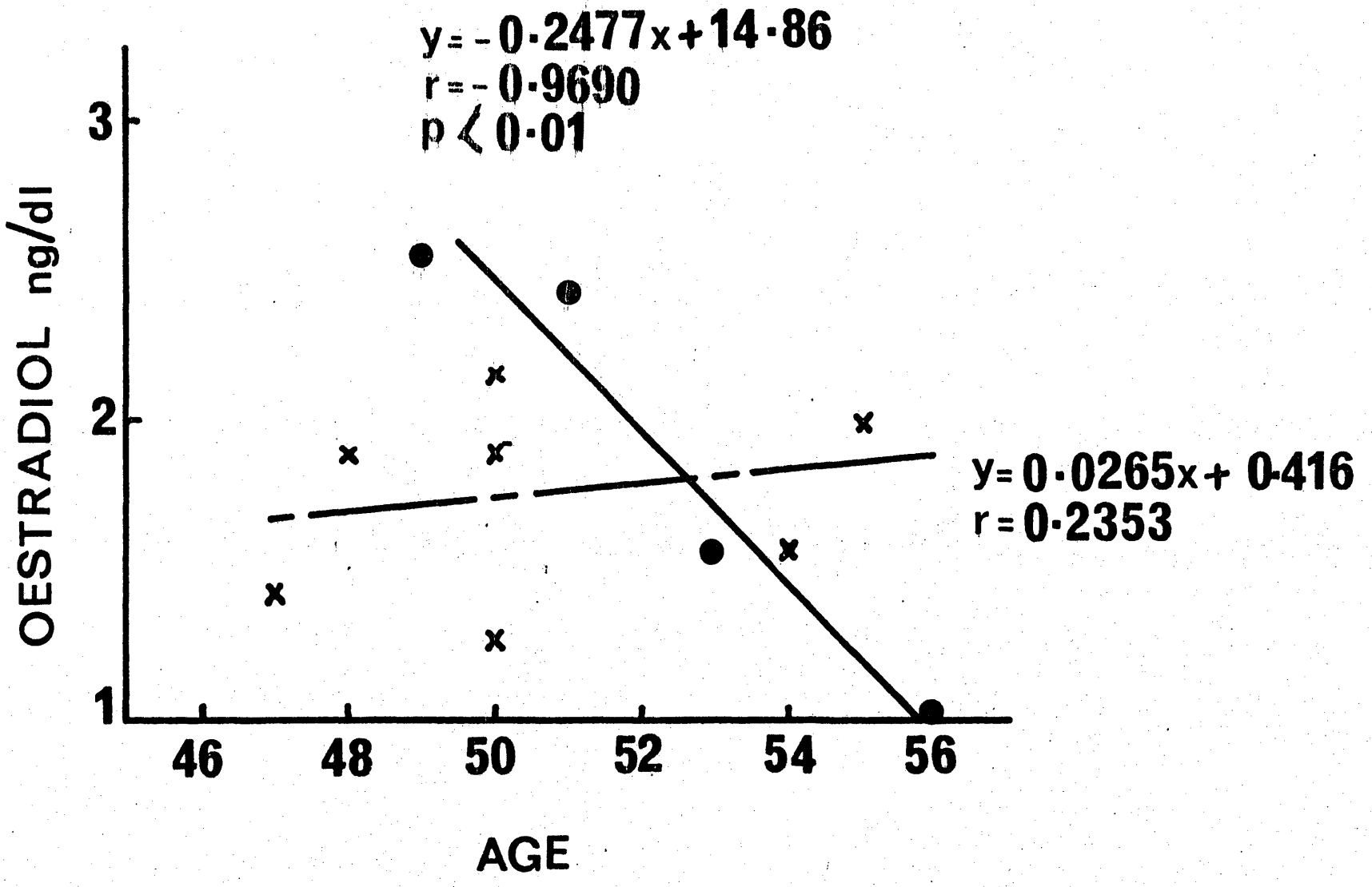

y = -0·2477x + 14·86
r = -0·9690
p < 0·01
y = 0·0265x + 0·416
r = 0·2353
OESTRADIOL ng/dl
3
2
1
46
48
50
52
54
56
AGE

Figure 2.16. Correlation of mean plasma oestradiol concentration and years since menopause or ovariectomy in four postmenopausal women (●) and seven ovariectomised women (×) after treatment with dexamethasone (1.5 mg daily) for one week.

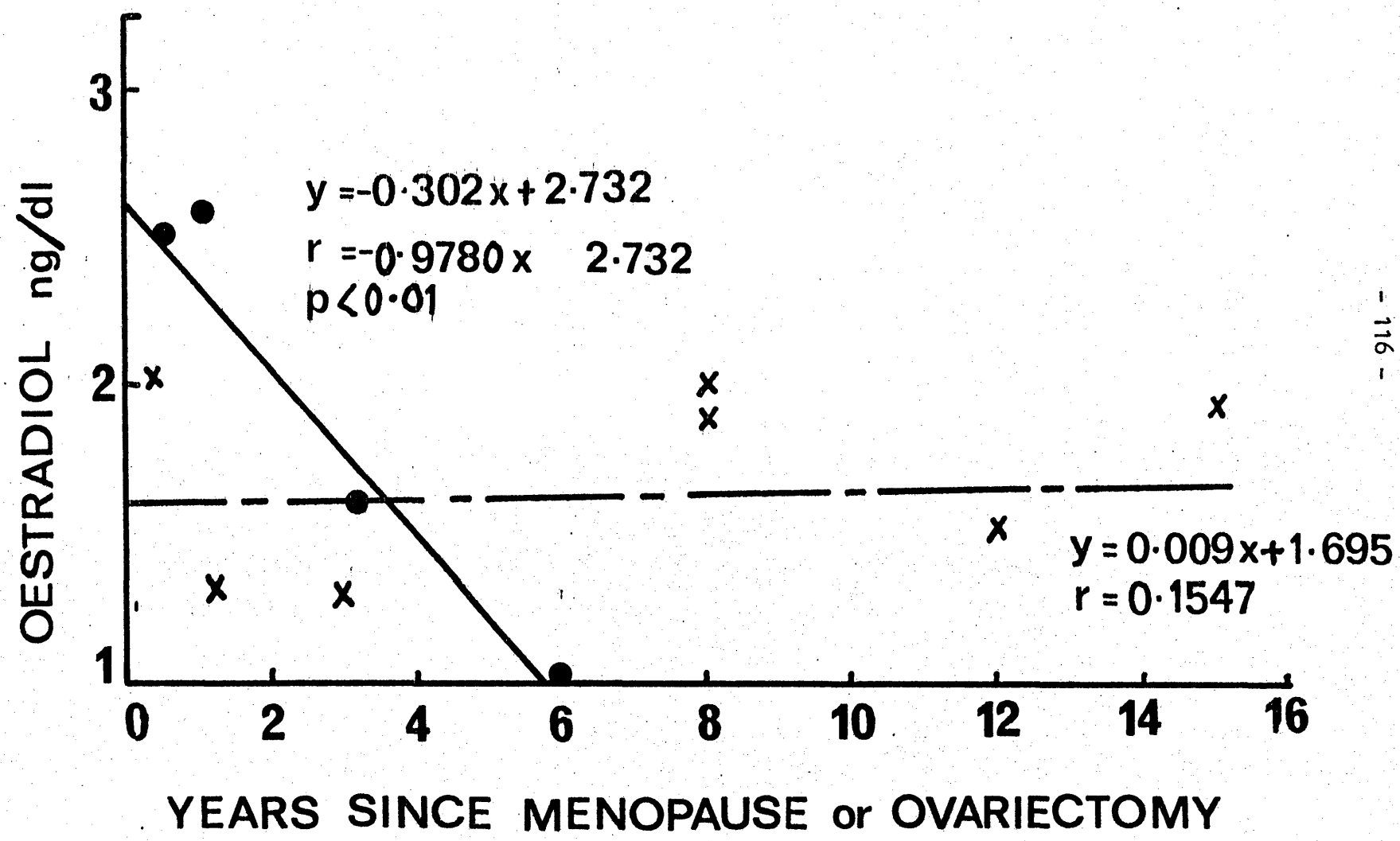
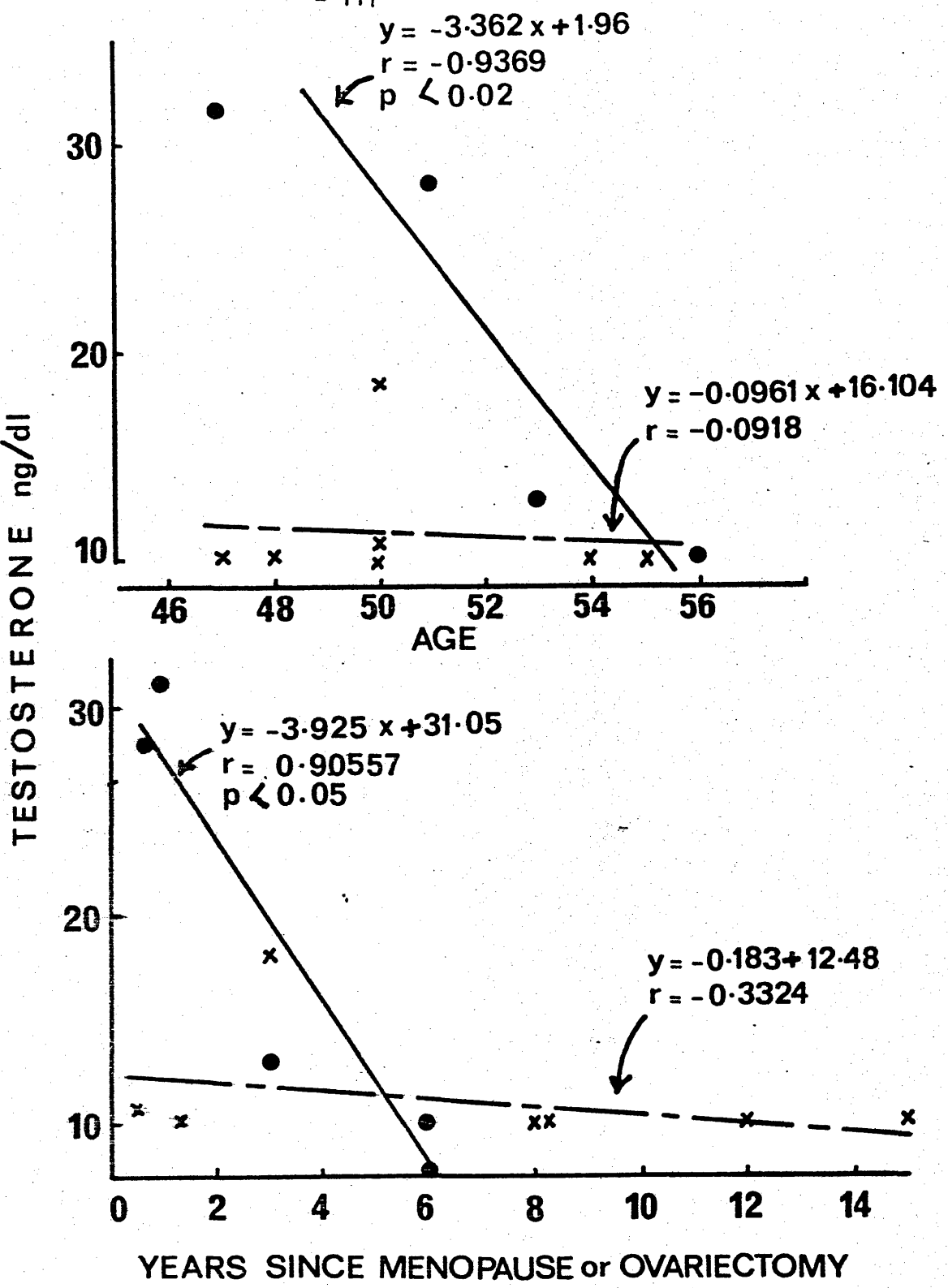

OESTRADIOL ng/dl
3
2
1
y =-0·302x+2·732
r =-0·9780x 2·732
p<0·01
y = 0·009x+1·695
r = 0·1547
0
2
4
6
8
10
12
14
16
YEARS SINCE MENOPAUSE or OVARIECTOMY

Figure 2.17. Correlations of mean plasma testosterone concentration and age and years since menopause in four postmenopausal women (●) and seven ovariectomised women (×) after treatment with dexamethasone (1.5 mg daily) for one week.


y = -3·362 x + 1·96
r = -0·9369
p < 0·02
y = -0·0961 x + 16·104
r = -0·0918
TESTOSTERONE ng/dl
30
20
10
46
48
50
52
54
56
AGE
y = -3·925 x + 31·05
r = 0·90557
p < 0·05
y = -0·183 + 12·48
r = -0·3324
30
20
10
0
2
4
6
8
10
12
14
YEARS SINCE MENOPAUSE or OVARIECTOMY

significant, the P values being < 0.02 and < 0.05 respectively. All the other correlations between the mean plasma oestrone, oestradiol, androstenedione and testosterone concentrations, and between these concentrations and the age, weight, percentage difference from the ideal weight and the interval since the menopause or ovariectomy, were not significant in either the postmenopausal or the ovariectomised women after the treatment with dexamethasone.

The FSH, LH and oestradiol concentrations in the women studied longitudinally are shown in Table 2.6. The progesterone concentrations were all below the 1 ng/dl limit of sensitivity of the assay.

DISCUSSION

Oestrogens: The mean plasma oestrone and oestradiol concentrations in the ovariectomised women in this study were similar to those in the postmenopausal women, a finding also observed by Saez et al. (1972), Marculis and Abraham (1976) and Vermeulen (1976). Thus the contribution of the ovary to the production of oestrogens after the menopause by the secretion of oestrogens or their androgenic prehormones was probably not significant. However, this conclusion might be invalid because there may have been abnormal peripheral production or metabolism of oestrogens that affected the plasma oestrogen concentrations in some of the ovariectomised women who were not strictly comparable to the postmenopausal women in this study. The heavier weight of the ovariectomised women compared to the postmenopausal women, may have affected the plasma oestrogen concentrations because it has been shown that the degree of aromatisation of androstenedione to oestrone in postmenopausal

Table 2.6. Plasma FSH, LH and oestradiol levels in a 51 year old woman (Subject No. 7) cylical piperazine oestrone sulphate 1.5mg daily between 17.1.76 and 28.5.76, and who had periods on 23.9.75, 4.4.76, 4.5.76, 6.6.76, 6.7.76, 8.8.76 and 1.10.76. Oestrogen therapy was recommenced on 29.11.76 because of a recurrence of flushes.

DATE	FSH ng/ml	LH ng/ml	E_2 ng/dl
16.11.76	268	28.7	3.8
24. 5.76	210	3.2	12.0
28. 6.76	72	8.2	3.1
26. 7.76	240	50.0	15.3
23. 8.76	80	6.4	4.5
11.10.76	32	4.5	13.1
20.10.76	29	3.2	3.2
29.11.76	210	17.5	3.0

women correlated with body weight (Siiteri and MacDonald, 1973; MacDonald, 1978), as did the plasma oestradiol concentration in normal postmenopausal women (Part 2 a). There was, however, no positive correlation of the plasma oestrogen concentrations with body weight or the percentage difference from ideal weight in the ovariectomised women in this study, although the correlation may have been affected by the fact that a few of the women were of about ideal weight. The indications for ovariectomy in the women had been carcinoma of the cervix and endometriosis, which have not been associated with abnormal oestrogen production or metabolism, and abnormal premenopausal uterine bleeding which, probably, had been primarily of ovarian origin and would therefore not have been affecting the plasma oestrogen concentrations in this study.

Although abnormal oestrogen production or metabolism in the ovariectomised women in this study may have occurred, the mean oestrogen concentrations were, nevertheless, within the range that has been reported by others. The mean oestradiol concentration of 3.2 $\pm$ 1.8 ng/dl in the ovariectomised women was similar to the 3.6 ng/dl concentration reported by Baird and Guevera (1969) although double that reported by Rader et al. (1973). Baird and Guevera (1969) also reported that the mean oestrone concentration in ovariectomised women was 11.6 ng/dl, a value twice that found in this study. The mean oestrone concentration in ovariectomised women studied by Marshall et al. (1977) was 3.0 ng/dl. The different values that have been reported may have resulted, at least in part, because investigators have studied women in whom ovariectomy had been indicated for different diseases of which some, such as

endometrial carcinoma, may have been associated with abnormal oestrogen metabolism (MacMahon, 1974).

The finding of similar plasma oestrogen concentrations in postmenopausal and ovariectomised women suggested that there was no direct ovarian secretion of oestrogens after the menopause, a conclusion which would be consistent with the findings of isotopic studies by Barlow et al. (1969). Moreover, Judd et al. (1974) and Greenblatt et al. (1976) found similar concentrations of oestrogens in peripheral and ovarian venous blood in postmenopausal women who were unlikely to have had abnormalities of oestrogen metabolism. In vitro studies by a number of workers (Savard et al., 1965; Rice and Savard, 1966; Plotz et al., 1967; Mattingly and Huang, 1969; Berman et al., 1973 and Wortman et al., 1975) have failed to demonstrate that the stroma of the postmenopausal ovary is capable of synthesising significant amounts of oestrogen.

The patterns of the plasma oestrone and oestradiol concentrations in the profiles of the ovariectomised women in this study were similar to those occurring in the postmenopausal women (as described in Part 2 a). Therefore, fluctuations in the plasma concentrations in postmenopausal women were considered unlikely to have resulted from episodic secretion of these oestrogens by the postmenopausal ovary. The possible role of adrenal and peripheral sources in the genesis of these asynchronous fluctuations will be described in subsequent parts of this thesis. The possibilities that these fluctuations may have resulted from methodological variations or fluctuations in the metabolic clearance rates of these oestrogens have been previously discussed (Part 2 a).

Sherman and Korenman (1975) and Sherman et al. (1976) showed

in perimenopausal women that plasma FSH and later LH concentrations increased as menstruation became irregular and that the levels, particularly of LH, fell when more regular cycles (some of which were ovulatory) recurred. The findings in the longitudinal study and particularly the markedly raised LH and oestradiol concentrations on 26.7.76 that are suggestive of the preovulatory peak, were consistent with Sherman's findings. However, in this study, blood samples for gonadotrophins, oestradiol and progesterone estimations were not obtained more frequently than monthly, and only one subject was studied. Thus, definite conclusions about ovulation and the length of the luteal phase in women who have had biochemical evidence suggestive of ovarian failure are not possible. However, this study demonstrates that significant follicular activity may still occur in older women with raised gonadotrophin concentrations, in whom less than one year has elapsed since their last period.

After the dexamethasone treatment, all the ovariectomised women in this study had almost undetectable plasma oestrone concentrations. A similar effect was found in a group of postmenopausal women (who were older than those in the present study) by Saez et al. (1972), Vermeulen (1976), Borkowski et al. (1977) and Santen et al. (1978) and also by Maroulis and Abraham (1976) after exclusion of one woman with presumptive biochemical evidence of ovulation. However, in the present investigation, plasma oestrone concentrations were undetectable only in the older postmenopausal women. If it is assumed that the dexamethasone treatment depleted the peripheral 'stores' of oestrone, as described below for oestradiol and in Parts 2 c and 2 d, then the plasma oestrone that was detected in the younger women may have been derived from the postmenopausal ovary.

Confirmation by further studies is however necessary because the number of women in this study was small. In premenopausal women, the amounts of circulating oestrone derived from direct follicular secretion and from peripheral conversion of oestradiol and androstenedione vary widely (Baird and Fraser, 1974), a finding which may explain the lack of a negative correlation between the plasma oestrone concentrations and the age or interval of amenorrhoea in the postmenopausal women.

In the ovariectomised women treated with dexamethasone in this study, the mean plasma oestradiol concentrations were, in almost all cases, less than 2 ng/dl, the level above which it has been shown that oestradiol concentrations can be reliably determined. As discussed more fully in Parts 2 c and 2 d, these results suggested that peripheral sources of plasma oestradiol in these women were depleted by the treatment with the dexamethasone for one week. If it is assumed that similar depletion occurred in postmenopausal women during the week's treatment with dexamethasone, then the finding in some of these women of plasma oestradiol concentrations $>$ 2 ng/dl suggested some production of oestradiol by the postmenopausal ovary. This conclusion was supported by the negative correlations of the plasma oestradiol concentration with the woman's age (Figure 2.15) and years since the menopause (Figure 2.16). Ovarian follicles may not become completely exhausted for several years after the menopause (Bloch, 1961), so this ovarian secretion of oestradiol may have originated in follicles rather than in the stroma. Although the amount of oestradiol secreted was insufficient to induce clinically apparent proliferative changes in genital tract epithelium, it was detectable by the biochemical techniques employed

in this study. Vermeulen (1976) reported that the mean plasma oestradiol concentration in women 4-10 years after the menopause was 1.2 ± 0.6 ng/dl after five days of treatment with dexamethasone. Maroulis and Abraham (1976) found that the plasma oestradiol concentration in postmenopausal women, most of whom were older than the subjects in this study, was undetectable, even before treatment with dexamethasone, and so a comparison of results is not possible. However, Maroulis and Abraham (1976) did find plasma oestradiol concentrations suggestive of recent ovulation in one other woman whose menopause apparently occurred two years previously. Ovulation and pregnancy have been reported as late as 11 years after the menopause (Sharman, 1962) and corpora lutea have been found in the ovaries of postmenopausal women (Forleo, 1973; Procope and Allercreutz, 1973). As the number of women in the present study was small, further studies are obviously required to determine more exactly the ovarian contribution to plasma oestradiol in women within two to three years of their menopause. As oestradiol is the principal secretory product of ovarian follicles and nearly all the subjects in this study were less than five years postmenopausal, the actual mean oestradiol concentration of 3.2 ± 1.2 ng/dl in the normal women may have been higher than if the concentrations had been determined in a group of older women in whom there was no possibility of oestradiol secretion by ovarian follicles. However, the younger women were deliberately investigated in this study because of the specific intention to determine the exact hormonal milieau of women at a time when they may be experiencing distressing symptoms of ovarian failure, and require oestrogen therapy. There is, however, no endocrine indication to conserve the ovary in women in whom more than 2-3 years has elapsed since their menopause, but because

ovulation may occur in women after there has been biochemical evidence of primary ovarian failure, perimenopausal women should be advised of contraception, probably until at least one year of amenorrhoea has occurred.

Androgens: Although mean concentrations of androstenedione in the 24 hour profiles in the ovariectomised women were similar to those occurring in the postmenopausal women, accurate conclusions about ovarian androstenedione production by this comparative technique were not possible because only six women were studied.

The similar testosterone concentrations in the 16 postmenopausal and 10 ovariectomised women in this study suggested that testosterone production by the postmenopausal ovary was insignificant, especially when compared with the adrenal contribution. As discussed earlier in this part, the groups of postmenopausal and ovariectomised women were, however, not similar with respect to age or weight, the latter of which has been found to affect the metabolic clearance rate of testosterone (Bardin and Lipsett, 1967). Because testosterone may also be derived by peripheral conversion of androstenedione (Baird et al., 1969), the plasma levels of testosterone in the ovariectomised women may also have been related to the levels of androstenedione whose production rate may be related to body weight (Calanog et al., 1977). As testosterone is largely cleared within the splanchnic circulation (Baird et al., 1969), the absence of the fallopian tubes, uterus and ovaries per se in the ovariectomised women was unlikely to have affected the clearance. The metabolic clearance rate of testosterone in ovariectomised women is lower than in premenopausal women (Abraham et al., 1969) but the clearance rate in postmenopausal women has not yet been reported. The mean testosterone concentrations in the ovariectomised women in this study,

were, however, mostly in the range reported by other investigators including Lobotsky et al. (1964) and Barberia and Thorneycroft (1974).

Although comparison of the peripheral concentrations in postmenopausal and ovariectomised women suggested that the postmenopausal ovary did not contribute significantly to testosterone production, the conclusion of some other studies has been to the contrary. Judd et al. (1974) showed that the mean peripheral testosterone concentration in postmenopausal women fell significantly ($P < 0.001$) after ovariectomy and concluded that the postmenopausal ovary must therefore contribute significantly to testosterone production. However, post-operatively half their women were prescribed medroxyprogesterone acetate therapy which has been shown to lower sex-hormone-binding globulin concentrations (Forest et al., 1968; Forest and Bertrand, 1972), and which might therefore have affected the plasma testosterone concentrations. The mean plasma testosterone concentrations before and after ovariectomy in the women studied by Judd et al. (1974) and who were not prescribed progestogen therapy were not significantly different. Judd et al. also assumed that the adrenal contribution to plasma testosterone concentrations was similar pre- and post-operatively, as the biochemical effects of the stress associated with an impending operation were not assessed

In another study, Judd et al. (1974) found a significant difference ($P < 0.01$) in the testosterone, but not androstenedione, concentration when samples from the ovarian vein were compared with those from peripheral veins in postmenopausal women. However, in that study, the correlation between peripheral and ovarian vein concentrations would not have been significant if four women with

endometrial carcinoma (or the premalignant adenomatous hyperplasia) had been excluded from the analysis (because they may have had abnormal androgen metabolism (Calanog et al., 1977)). Judd et al. also found difficulty in obtaining blood from the ovarian veins, presumably because the rate of blood flow was low. Thus, although the gradient studies may have confirmed the findings of in vitro studies which showed that the postmenopausal ovary can secrete testosterone, the contribution to plasma testosterone production remains uncertain and is likely to be small. Clearly, further studies are required.

The plasma androstenedione and testosterone concentrations after dexamethasone were not significantly different in the postmenopausal and ovariectomised women. If it is assumed that the dexamethasone treatment depleted the peripheral sources of plasma androgens, as described above for the oestrogens, then the results of this study suggest that there may have been some secretion of testosterone, but not androstenedione, by the ovaries of the younger women (Figure 2.17). However, further studies are required to determine whether testosterone (and oestrone and oestradiol) in women within 2-3 years of the menopause may be secreted by follicles still remaining in the ovary. As also reported by Vermeulen (1976), the mean androstenedione concentrations in the ovariectomised and postmenopausal women after treatment with dexamethasone were < 28 ng/dl, which is a level near the limits of detection of the assay. Thus and in agreement with isotope studies of postmenopausal and ovariectomised women by Reed (1978) it may be concluded that in postmenopausal women, the ovary did not contribute significantly to androstenedione production. However,

the results of these studies are not in agreement with those of Borkowski et al. (1977) who found plasma androstenedione concentrations 50 ng/dl in postmenopausal and ovariectomised women, even after six weeks of treatment with dexamethasone. However, all the women in their study had breat carcinoma which may be associated with abnormal steroid production or metabolism (Bulbrook and Greenwood, 1957; Bulbrook et al., 1958; Siiteri et al., 1974).

Part 2 c) THE CONTRIBUTION OF THE ADRENAL CORTEX TO PLASMA STEROIDS

As discussed in the introduction (Part 1) and deduced from the study of the contribution of the postmenopausal ovary to plasma steroids (Part 2 b), the adrenal cortex is the major source of plasma steroids after the menopause. The intensive hormone profile studies of the postmenopausal and ovariectomised women in Parts 2 a and 2 b showed that only cortisol and androstenedione concentrations fluctuated synchronously with a diurnal rhythm and thus, that ACTH regulated the acute secretion of only these two steroids. The aim of this study was to further examine the role of the adrenal cortex, and particularly its major trophic hormone ACTH, in the production of plasma steroids after the menopause, by determining the effects on plasma hormone concentrations of suppression of endogenous ACTH production by dexamethasone and of infusion of tetracosactrin. It has been assumed in this study that there was no significant contribution to steroid production by the postmenopausal ovary, and therefore no distinction has been drawn between different effects of ACTH and dexamethasone in postmenopausal and ovariectomised women. However, and as described in Part 2 b, steroid secretion by the ovary may have occurred in those women in whom less than two years had elapsed since their menopause.

METHOD

a) Effects of Dexamethasone Treatment

Eight postmenopausal or ovariectomised women were studied on two occasions, a control night and a treatment night. During both sampling periods, blood samples were obtained every 30 minutes for the determination of plasma cortisol, androstenedione, testosterone, oestrone and oestradiol concentrations. On the control night, the

samples were collected for 12-14 hours commencing at 2200-2400 hours. Five women were then studied on the following night when dexamethasone 2 mg and 0.5 mg was administered orally at 2400 hours and 0800 hours respectively, and during which blood samples were obtained from about 2300 hours until at least 0830 hours. The clinical details of these five women are summarised in Table 2.7. The other three women were discharged after the control night and readmitted seven days later after they had taken dexamethasone (1.5 mg daily in a divided dose). Blood samples were then obtained between 2400 hours and 0830 hours after which ACTH or androstenedione was infused (as discussed below). The clinical details of these three women are summarised in Table 2.8. There was no significant difference between the age, years since the menopause or ovariectomy, body weight or percentage difference from the ideal body weight (Geigy, 1970) between the five women studied after one dose of dexamethasone and the three women studied after one week of treatment with dexamethasone.

The indications for the pelvic clearance in the ovariectomised women had been carcinoma of the cervix (2 women), abnormal uterine bleeding (2 women) and endometriosis (1 woman): the other three women (Subject Nos. 6, 16 & 17) were postmenopausal.

b) Effects of Infusions of Tetracosactrin

Tetracosactrin was infused at a constant rate of 3 µg/hour through a scalp vein needle in a forearm vein for six hours beginning at 0830 hours in five of the women described above who had been prescribed dexamethasone for one day (Subject Nos. 6, 20 & 26) or for one week (Subject Nos. 7 & 19). Plasma cortisol, androstenedione, testosterone, oestrone and oestradiol concentrations were determined in blood samples that were obtained every 30 minutes

Table 2.7. Clinical details and mean ± SD cortisol (F), androstenedione (A), testosterone (T), oestrone (E_1) and oestradiol (E_2) concentrations in blood samples obtained every 30 minutes between 0430 and 0830 on a control morning (Pre-Dex) and on the following morning after treatment with dexamethasone 2mg at 2400h (Post-Dex) and, in three subjects during the last four hours of a six hour infusion of tetracosactrin (ACTH). (Significance of differences from Pre-Dex levels: *** p = <0.001, ** p = <0.01, * p = <0.05. (M) denotes the Mann Whitney U test).

NO	AGE	YEARS MP/OV	WT (Kg)	% DIFF	SAMPLING PERIOD	F µg/dl	A ng/dl	T ng/dl	E_1 ng/dl	E_2 ng/dl
					Pre-Dex	20.8 ± 5.3	190.2 ±53.1	65.9 ± 8.9	5.8 ± 1.8	5.0 ± 0.9
6	54	1.9	61	+12	Post-Dex	2.0 ± 0.7***(M)	37.8 ± 8.8***(M)	43.5 ± 6.2***	2.7 ± 1.3***	4.3 ± 0.4*
					ACTH	34.4 ± 1.6***(M)	297.5 ±46.0***	82.3 ± 6.4***	10.1 ± 1.6***	5.7 ± 1.5
					Pre-Dex	13.5 ± 6.7	102.7 ±41.7	31.7 ± 7.2	2.6 ± 0.4	2.3 ± 0.3
20	42	4.0 (OV)	72	+14	Post-Dex	1.1 ± 0.2***(M)	<26.6***(M)	11.5 ± 2.3***	<1.0***(M)	1.8 ± 0.3
					ACTH	42.5 ± 8.2***	183.3 ±22.5***	28.1 ± 4.6	2.4 ± 0.7	3.0 ± 0.5
					Pre-Dex	8.9 ± 6.9	75.5 ±14.0	39.4 ±10.9	7.1 ± 1.1	2.0 ± 0.6
26	53	15.0 (OV)	74	+19	Post-Dex	<1.0***(M)	27.2 ± 1.2***(M)	32.0 ± 7.7	4.7 ± 0.9***	2.4 ± 0.5
					ACTH	32.7 ± 3.4***	104.2 ±13.8***	49.6 ±15.9	2.8 ± 1.2***	3.0 ± 0.7*
					Pre-Dex	8.6 ± 1.8	115.0 ±28.6	23.6 ± 2.1	3.8 ± 0.9	5.4 ± 0.7
16	49	0.9	71	+ 9	Post-Dex	2.2 ± 3.1***	30.5 ± 3.1***(M)	23.6 ± 2.1	1.9 ± 0.8***	5.4 ± 1.0
					Pre-Dex	10.1 ± 4.6	104.2 ±28.6	12.6 ± 4.5	6.4 ± 2.2	2.3 ± 0.9
25	42	2.0 (OV)	64	+16	Post-Dex	<3.0***(M)	37.0 ± 5.0***(M)	15.0 ± 5.7	3.1 ± 1.7*	2.0 ± 0.4

Table 2.8. Clinical details and mean ± SD steroid concentrations in blood samples obtained every 30 minutes between 0430 and 0830 hours on a control morning (Pre-Dex) and a morning one week later after treatment with dexamethasone 1.5mg daily (Post-Dex) and, in two subjects during the last four hours of a six hour infusion of tetracosactrin (ACTH). (Significance of difference from Pre-Dex levels: *** $p = <0.001$, ** $p = < 0.01$. (M) is Mann Whitney U test)

NO	AGE	YEARS MP/OV	WT (Kg)	% DIFF	SAMPLING PERIOD	F µg/dl	A ng/dl	T ng/dl	E_1 ng/dl	E_2 ng/dl
7	53	3.0	63	+ 1.0	Pre-Dex	18.0 ± 3.4	62.5 ± 18.9	21.2 ± 5.0	4.4 ± 1.2	3.3 ± 0.6
					Post-Dex	<1.0*** (M)	<26.6*** (M)	11.0 ± 1.4	2.9 ± 0.5**	1.7 ± 0.7***
					ACTH	19.0 ± 0.7	52.4 ± 10.7	13.1 ± 3.1***	4.0 ± 1.1	1.3 ± 0.3***
19	50	3.0 (OV)	63	+ 2.4	Pre-Dex	16.5 ± 5.2	94.2 ± 18.9	40.4 ± 6.7	4.6 ± 0.6	3.3 ± 0.8
					Post-Dex	<1.0*** (M)	<26.6*** (M)	16.8 ± 6.3***	1.0 ± 0.1*** (M)	1.2 ± 0.3***
					ACTH	18.4 ± 2.6	51.4 ± 8.4***	24.1 ± 7.5***	3.3 ± 0.7***	2.0 ± 0.4***
21	47	1.1 (OV)	61	+11.0	Pre-Dex	10.5 ± 3.4	88.7 ± 28.9	28.8 ± 3.2	8.2 ± 0.8	3.2 ± 0.4
					Post-Dex	<1.0*** (M)	<26.6*** (M)	<10.0*** (M)	2.0 ± 1.0***	1.4 ± 0.4***

during the infusion and, except for Subject No. 6, also for four hours after the infusion.

Full clinical and sampling details of all the women in this study are given in Appendix IV.

RESULTS

a) Effects of Dexamethasone

The hormone profiles of all the subjects are shown in Figures 2.18-2.25. The hormone levels, patterns and interrelationships in these women on the control night were similar to those observed in the more intensive hormone profiles (24 hour) of the four postmenopausal women (Figures 2.1-2.4, Part 2 a) and the two ovariectomised women (Figures 2.13 and 2.14, Part 2 b) and, in particular, the plasma concentrations of only cortisol and androstenedione fluctuated synchronously and with a diurnal rhythm. The mean plasma steroid concentrations during the early morning surge of cortisol and androstenedione, between 0430 and 0830 hours, are shown in Table 2.7 for the five women subsequently treated with a single dose of dexamethasone, and in Table 2.8 for the other three women subsequently treated with dexamethasone for one week.

The hormone profiles of the five women to whom the 2 mg dose of dexamethasone was administered at 2400 hours on the night following the 'control' night, are shown in Figures 2.18-2.22. In all five women, the early morning surge of cortisol and androstenedione was suppressed by the administration of the dexamethasone. The other steroids showed similar levels and fluctuations to those that occurred on the 'control' night. The mean plasma steroid concentrations in the blood samples obtained between 0430 and 0830 hours from each of these women are also shown

Figure 2.18. Plasma steroid concentrations in blood samples obtained from an ovariectomised woman (Subject No. 25) every 30 minutes on a control night, and on the following night when dexamethasone (Dex) was administered.

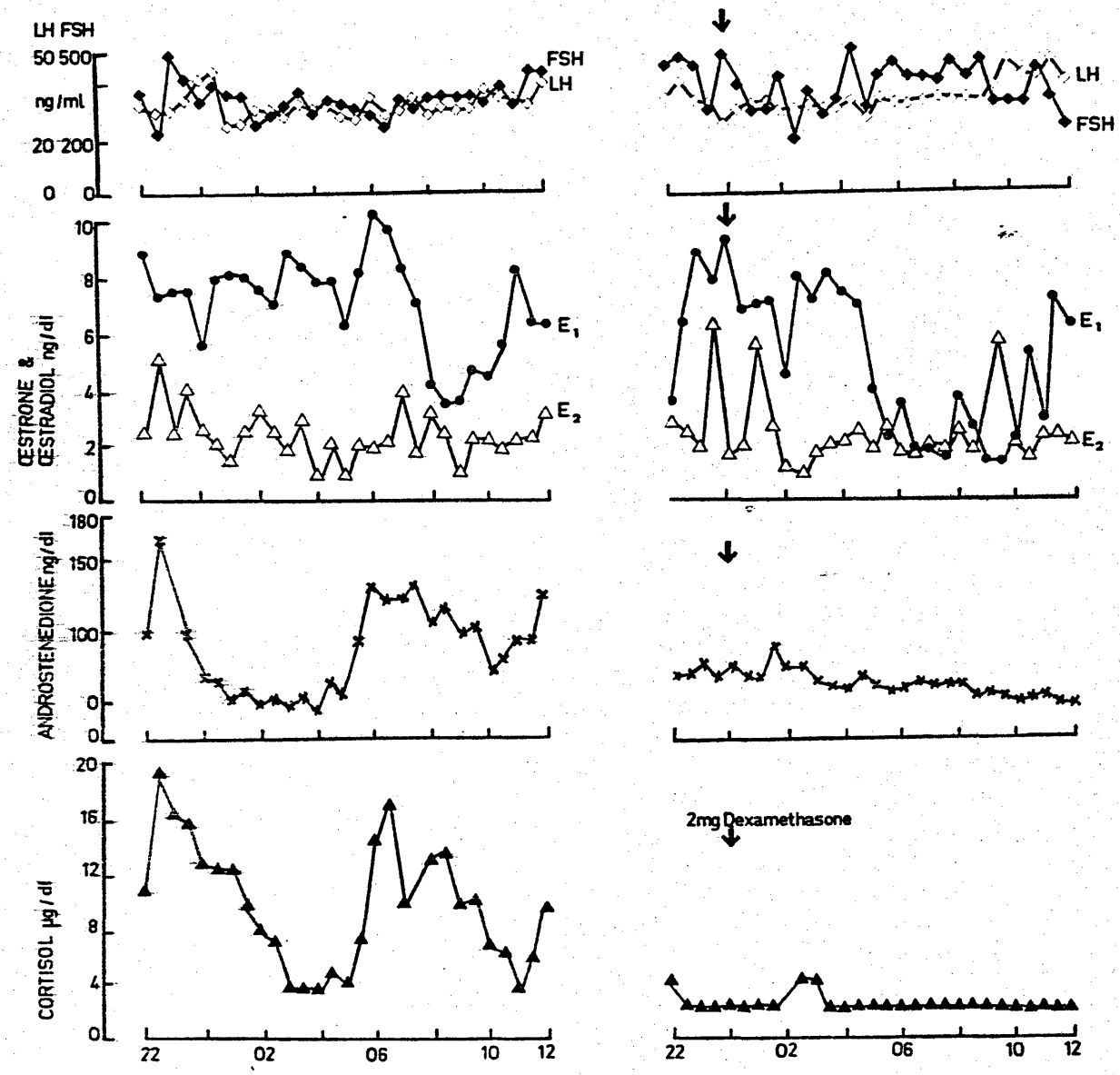

LH FSH
50 500
ng/ml
20 200
0 0
FSH
LH
LH
FSH
10
8
6
4
2
0
ŒSTRONE &
ŒSTRADIOL ng/dl
E1
E2
E1
E2
180
150
100
0
0
ANDROSTENEDIONE ng/dl
20
16
12
8
4
0
CORTISOL µg/dl
2mg Dexamethasone
22
02
06
10
12
22
02
06
10
12

Figure 2.19. Plasma steroid concentrations in blood samples obtained from a postmenopausal woman (Subject No. 16) every 30 minutes on a control night, and on the following night when dexamethasone (Dex) was administered.

0.9 yrs since MP
Age 64

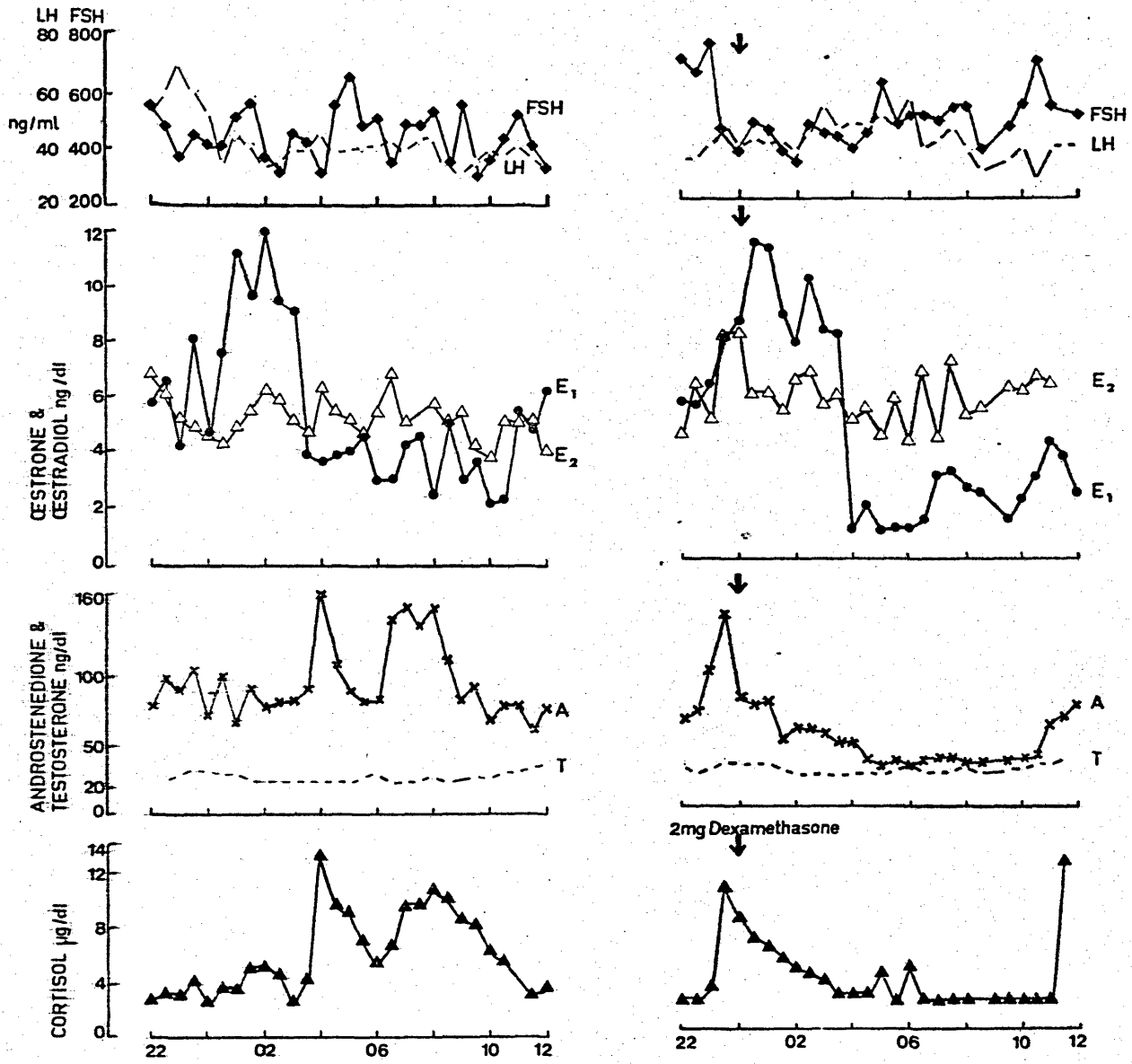

LH FSH
80 800
60 600
ng/ml
40 400
20 200
FSH
LH
ŒSTRONE & ŒSTRADIOL ng/dl
12
10
8
6
4
2
0
E1
E2
ANDROSTENEDIONE & TESTOSTERONE ng/dl
160
100
50
20
0
A
T
CORTISOL µg/dl
14
12
8
4
0
22
02
06
10
12
2mg Dexamethasone

Figure 2.20. Plasma steroid concentrations in blood samples obtained from an ovariectomised woman (Subject No. 20) every 30 minutes on a control night, and on the following night when dexamethasone (Dex) was administered, and tetracosactrin (ACTH) was infused at a rate of 3 µg/hour for six hours.

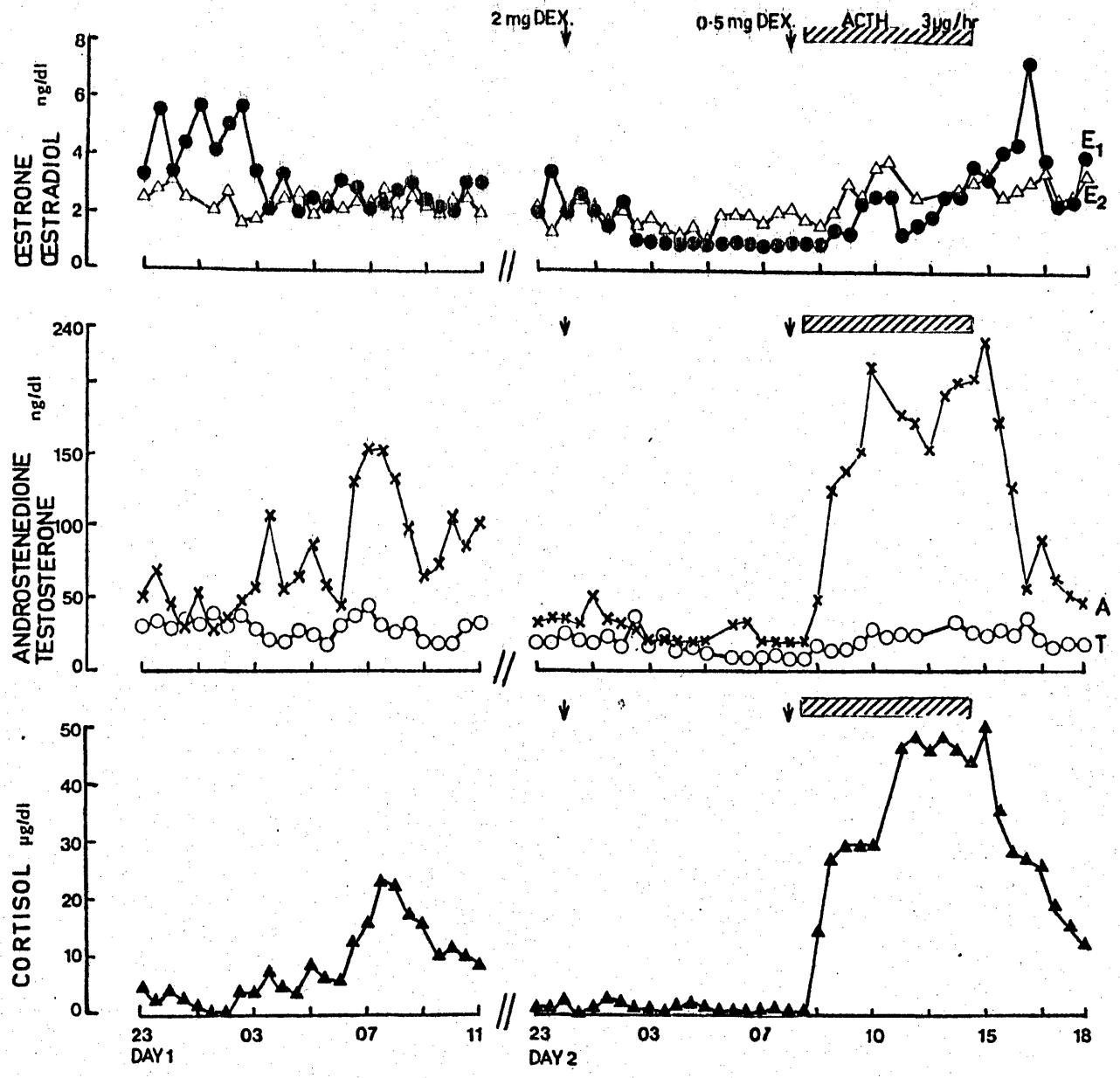

2 mg DEX.
0·5 mg DEX.
ACTH 3µg/hr
ŒSTRONE
ŒSTRADIOL
ng/dl
8
6
4
2
0
E1
E2
ANDROSTENEDIONE
TESTOSTERONE
ng/dl
240
150
100
50
0
A
T
CORTISOL µg/dl
50
40
30
20
10
0
23
03
07
11
DAY 1
23
03
07
10
15
18
DAY 2

Figure 2.21. Plasma steroid concentrations in blood samples obtained from an ovariectomised woman (Subject No. 26) every 30 minutes on a control night, and on the following night when dexamethasone (Dex) was administered and tetracosactrin (ACTH) was infused for six hours at a rate of 3 µg/hour.

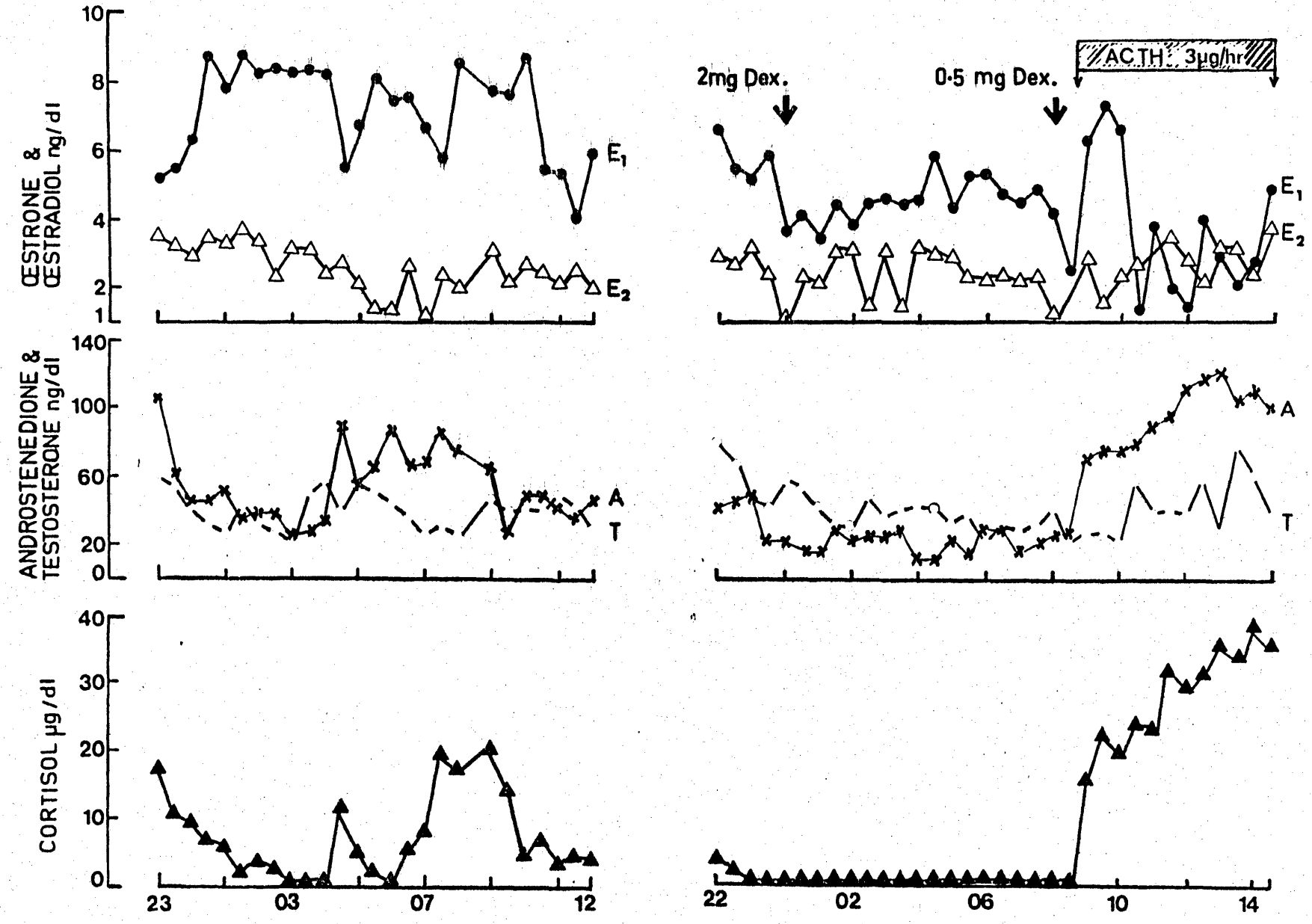

ŒSTRONE & ŒSTRADIOL ng/dl
10
8
6
4
2
1
E1
E2
2mg Dex.
0·5 mg Dex.
ACTH 3µg/hr
ANDROSTENEDIONE & TESTOSTERONE ng/dl
140
100
60
20
0
A
T
CORTISOL µg/dl
40
30
20
10
0
23
03
07
12
22
02
06
10
14

Figure 2.22. Plasma steroid concentrations in blood samples obtained from a postmenopausal woman (Subject No. 6) every 30 minutes on a control night, and on the following night when dexamethasone (Dex) was administered, and tetracosactrin (ACTH) was infused at a rate of 3 μg/hour for six hours.

1.9 yrs MP

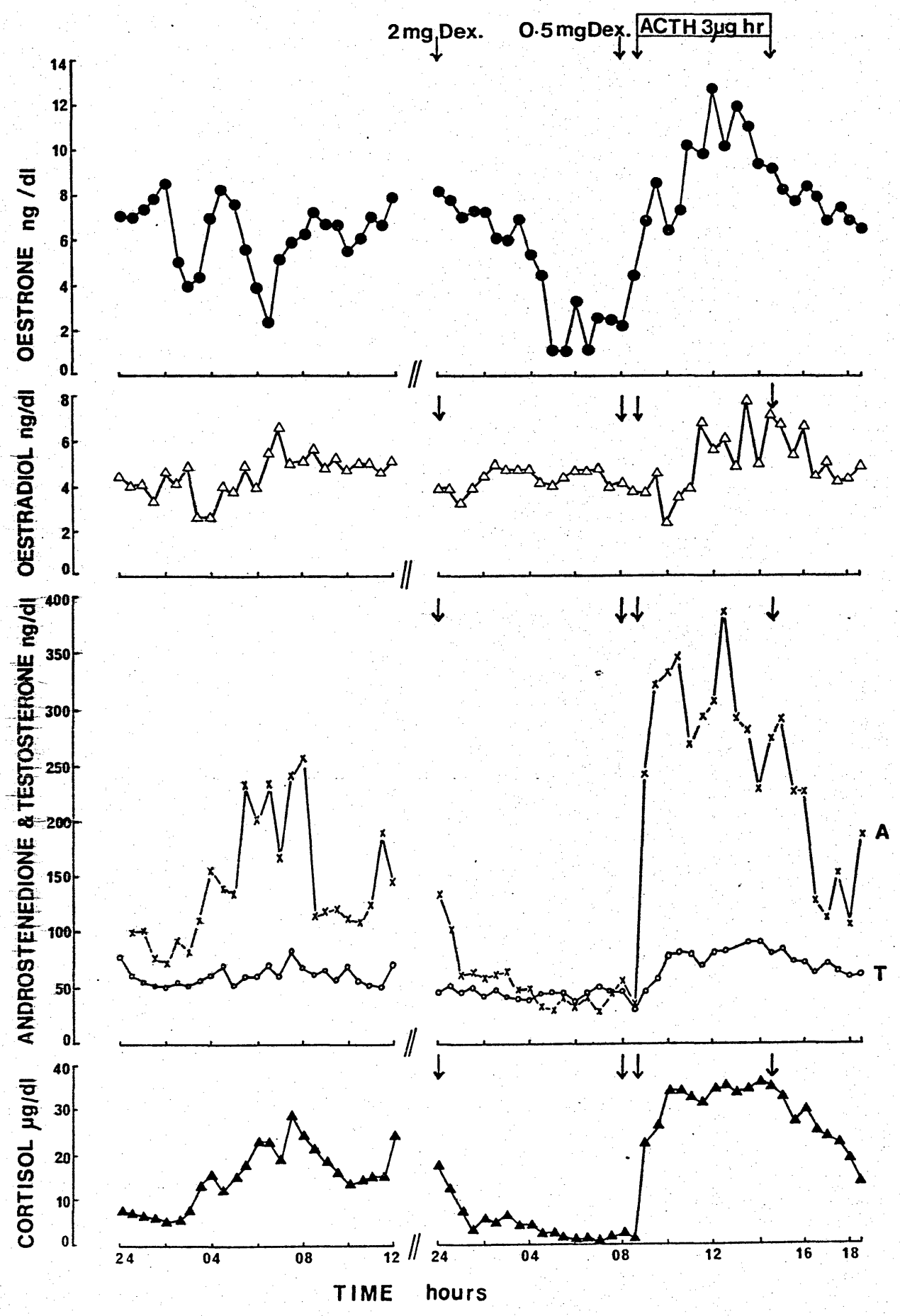

2mg Dex.
0·5mg Dex.
ACTH 3µg hr
OESTRONE ng /dl
OESTRADIOL ng/dl
ANDROSTENEDIONE & TESTOSTERONE ng/dl
A
T
CORTISOL µg/dl
24
04
08
12
16
18
TIME hours

Figure 2.23. Plasma steroid concentrations in blood samples obtained from an ovariectomised woman (Subject No. 19) on a control night, and then one week later after treatment with dexamethasone (Dex), 1.5 mg daily, when tetracosactrin (ACTH) was also infused at a rate of 3 μg/hour.

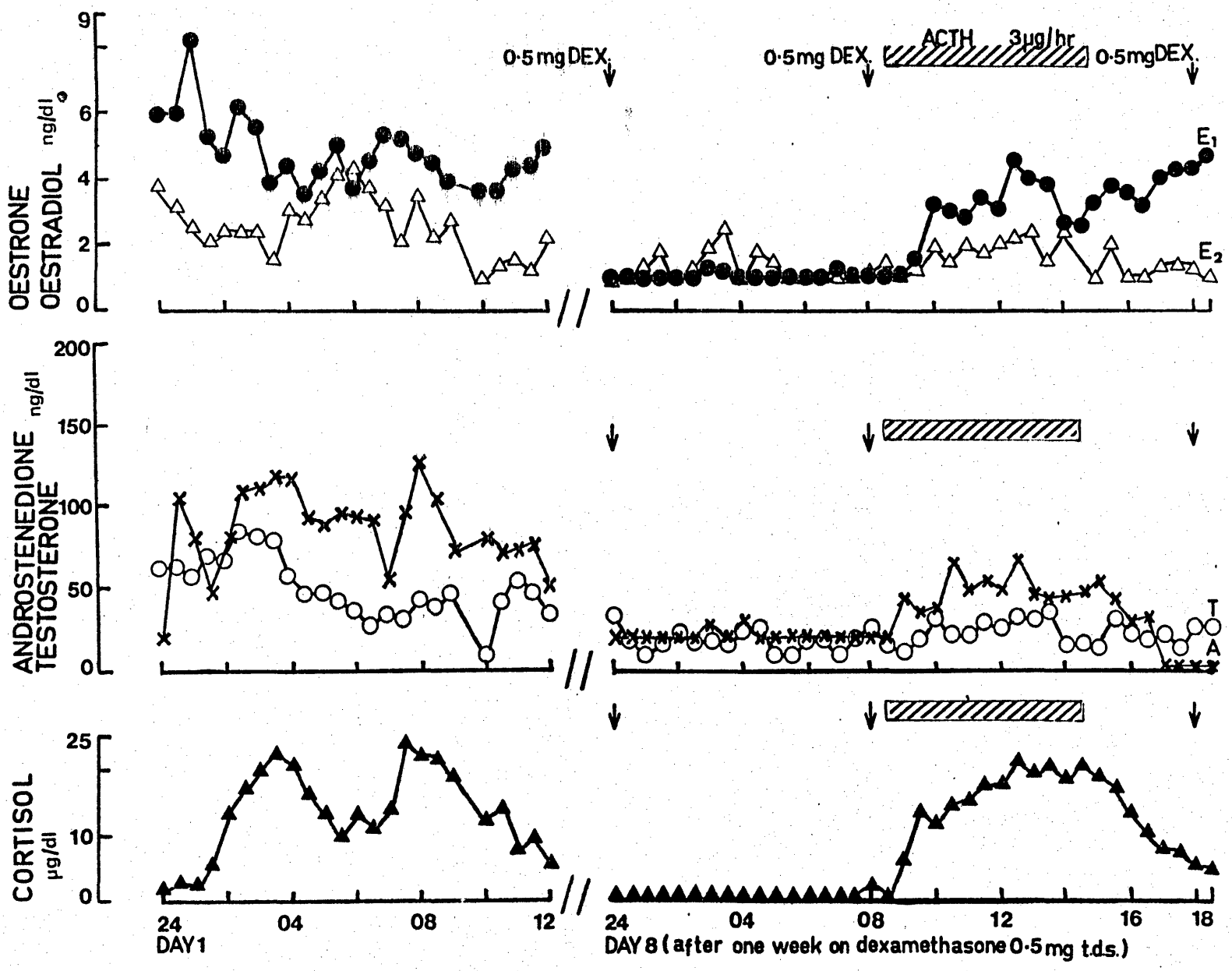

0·5mg DEX.
0·5mg DEX.
ACTH 3μg/hr
0·5mg DEX.
OESTRONE
OESTRADIOL ng/dl
E1
E2
ANDROSTENEDIONE ng/dl
TESTOSTERONE
T
A
CORTISOL μg/dl
24
04
08
12
16
18
DAY 1
DAY 8 (after one week on dexamethasone 0·5 mg t.d.s.)

Figure 2.24. Plasma steroid concentrations in blood samples obtained from a postmenopausal woman (Subject No. 7) on a control night, and then one week later after treatment with dexamethasone (Dex) 1.5 mg daily, when tetracosactrin (ACTH) was also infused at a rate of 3 μg/hour.

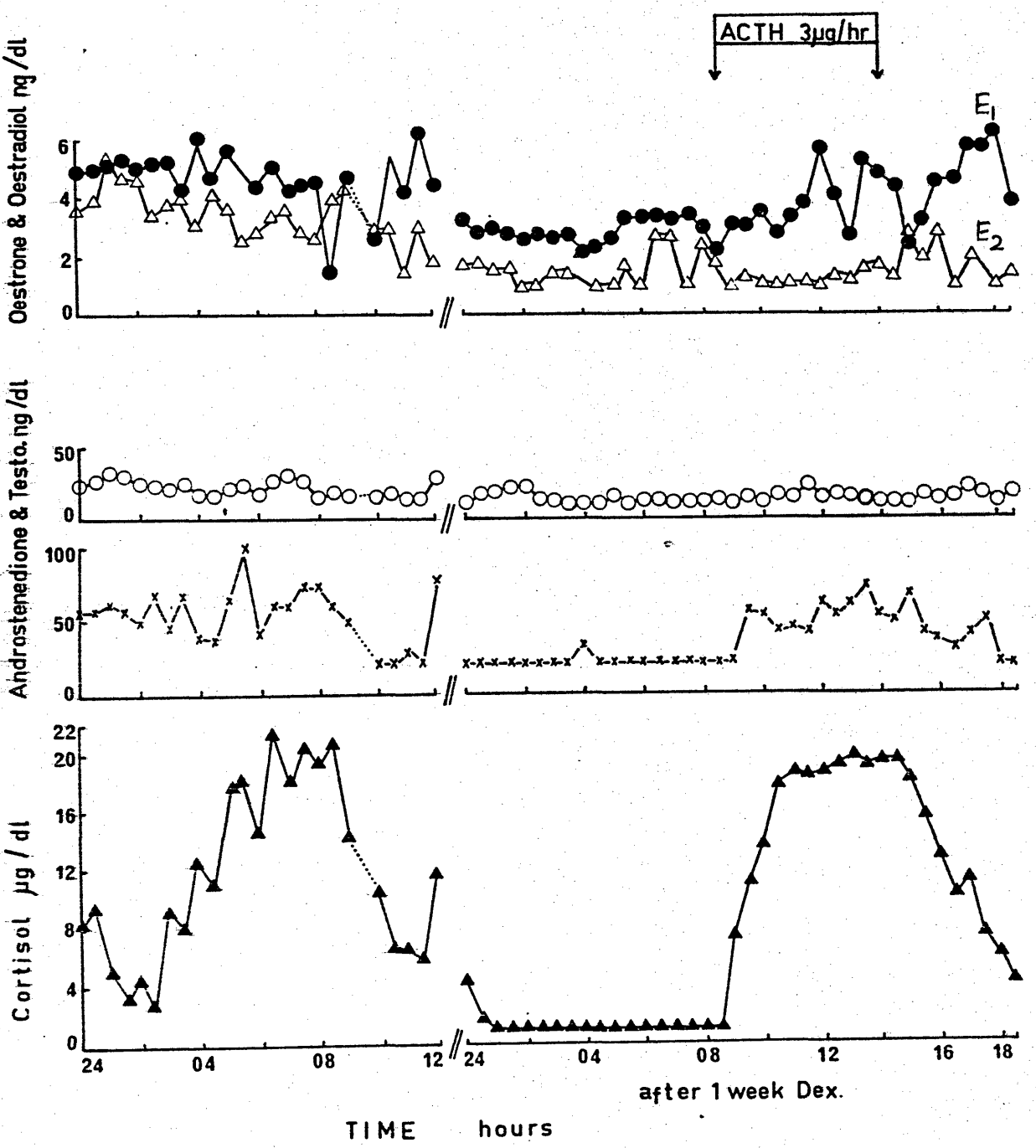
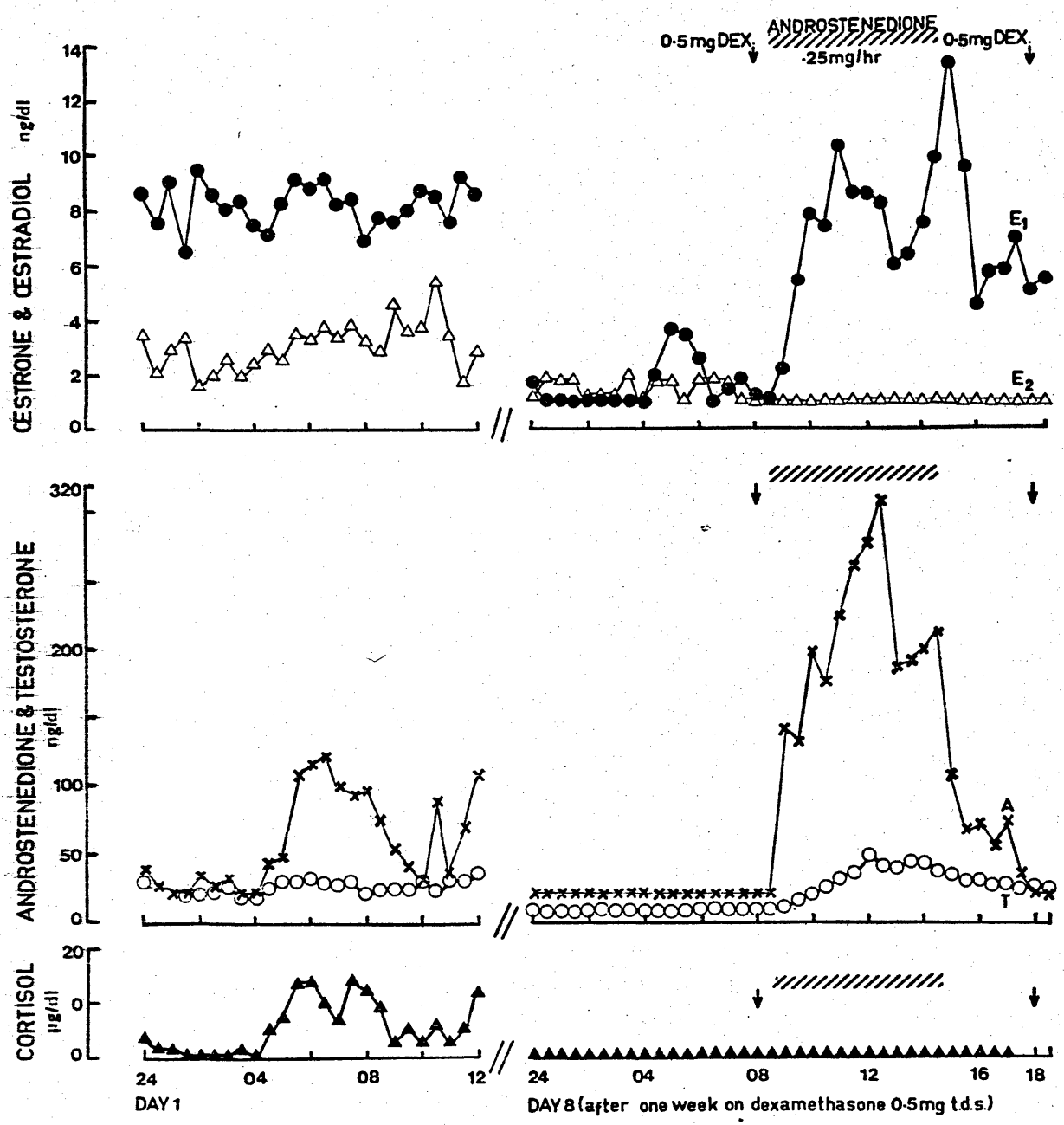

ACTH 3µg/hr
E1
E2
Oestrone & Oestradiol ng/dl
6
4
2
0
Androstenedione & Testo. ng/dl
50
0
100
50
0
Cortisol µg/dl
22
20
16
12
8
4
0
24
04
08
12
24
04
08
12
16
18
after 1 week Dex.
TIME hours

Figure 2.25. Plasma steroid concentrations in blood samples obtained from an ovariectomised woman (Subject No. 21) on a control night and one week later after treatment with dexamethasone (Dex) 1.5 mg daily, when androstenedione was also infused at a rate of 250 μg/hour for six hours.

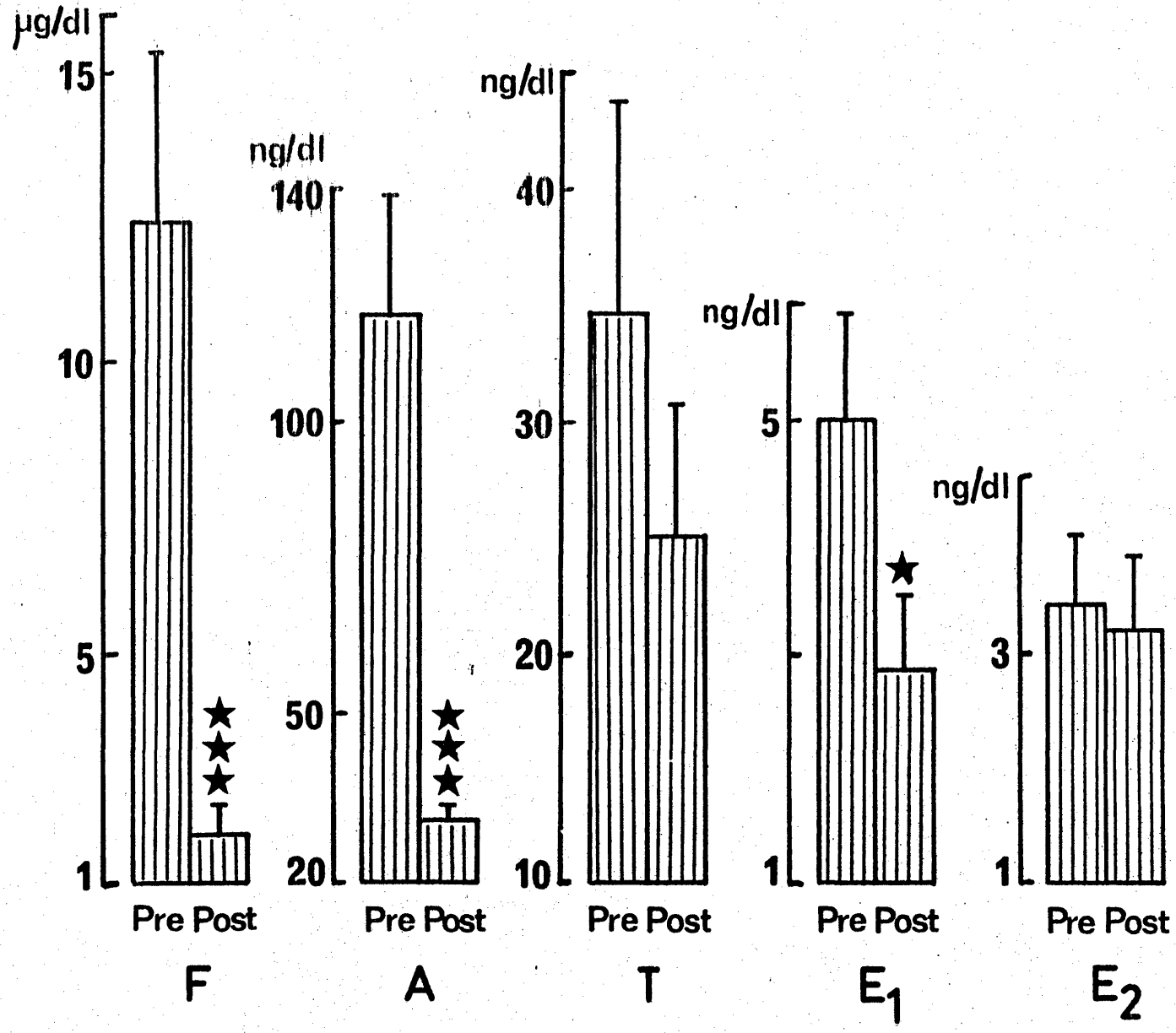

ŒSTRONE & ŒSTRADIOL ng/dl
14
12
10
8
6
4
2
0
0·5mg DEX
ANDROSTENEDIONE
·25mg/hr
0·5mg DEX
E1
E2
ANDROSTENEDIONE & TESTOSTERONE ng/dl
320
200
100
50
0
A
T
CORTISOL µg/dl
20
0
0
24
04
08
12
DAY 1
24
04
08
12
16
18
DAY 8 (after one week on dexamethasone 0·5mg t.d.s.)

in Table 2.7. The mean cortisol and androstenedione concentrations were usually near the limits of detection of the assay, and were significantly lower than the mean concentrations during the control night. In two women, mean plasma testosterone concentrations were also significantly lower ($P < 0.001$) than on the 'control' night but the testosterone levels in the other three women were not affected by the dexamethasone treatment. Although in four of the five women plasma oestrone concentrations were also significantly lower than on the'control' night, unlike cortisol and androstenedione, the levels were usually only halved, and were not suppressed to near the limits of detection of the assay. The oestradiol concentrations after the dexamethasone were similar to those on the 'control' night except for Subject No. 20 (Figure 2.20) in whom the mean level of 1.8 ± 0.3 ng/dl after the dexamethasone was significantly lower ($P < 0.01$) than the level of 2.3 ± 0.3 ng/dl on the 'control' night.

The mean concentrations of cortisol, androstenedione, testosterone, oestrone and oestradiol in the blood samples obtained between 0430 and 0830 hours in the group of five women before and after the single dose of dexamethasone are shown in Figure 2.26. Only cortisol and androstenedione concentrations were significantly lower than the concentrations on the control night. Although mean plasma testosterone and oestrone concentrations on the treatment night were lower than on the control night, the differences were not significant.

The hormone profiles of the three women treated with dexamethasone for one week are shown in Figures 2.23-2.25. The plasma concentrations of all the steroids after the dexamethasone treatment were mostly near the limits of detection of the assays except in one

Figure 2.26. Mean ± SEM cortisol (F), androstenedione (A), testosterone (T), oestrone (E_1) and oestradiol (E_2) concentrations in nine blood samples obtained between 0430 and 0830 hours from five women before (Pre) and after (Post) treatment with dexamethasone 2 mg at 2400 hours. Levels of significance of differences between pre- and post- concentrations are denoted * $P < 0.05$, *** $P < 0.001$.

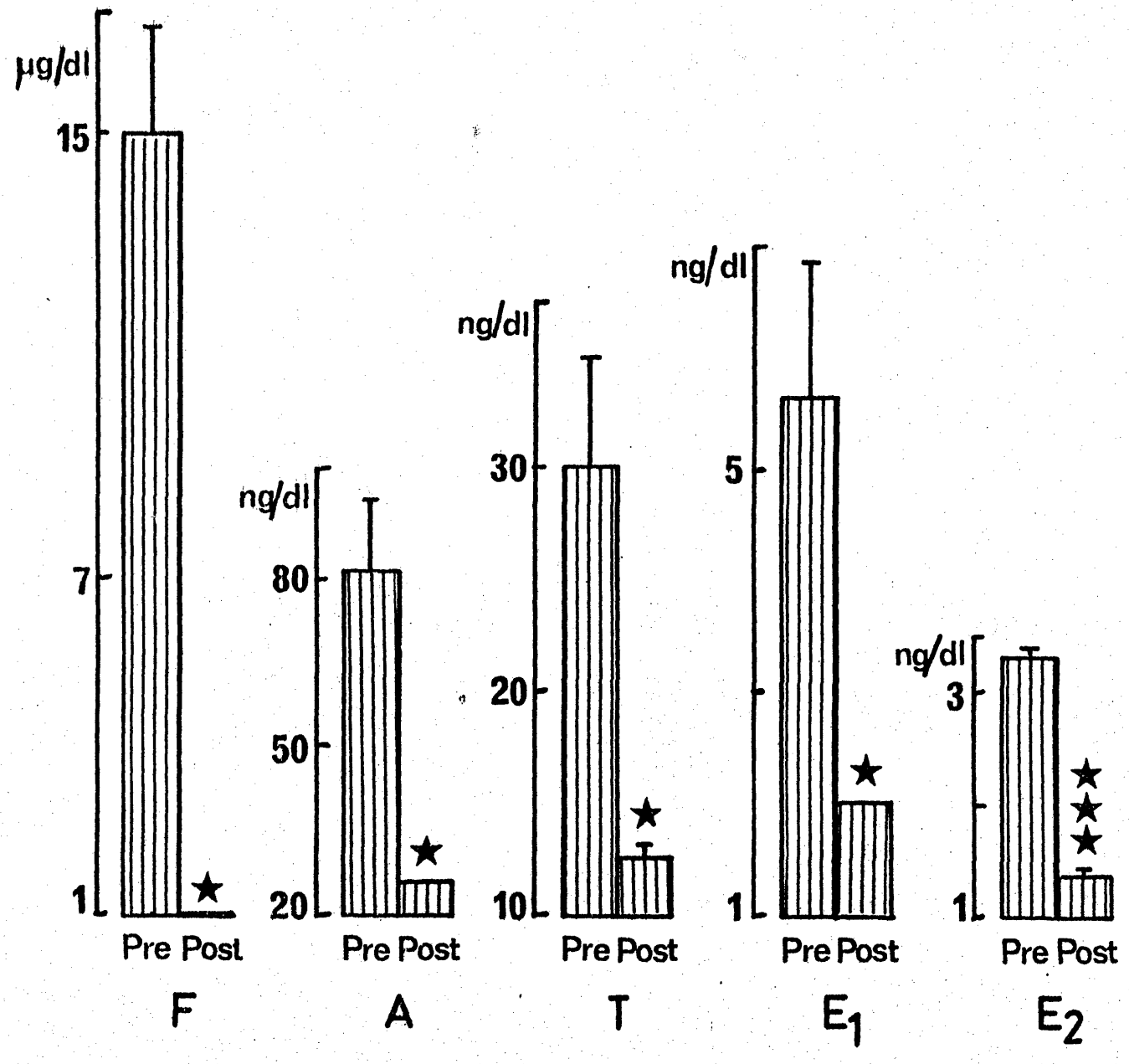

µg/dl
15
10
5
1
Pre Post
F
ng/dl
140
100
50
20
Pre Post
A
ng/dl
40
30
20
10
Pre Post
T
ng/dl
5
1
Pre Post
E1
ng/dl
3
1
Pre Post
E2

postmenopausal woman (Subject No. 7, Figure 2.24). Although oestrone was detectable in this subject after the dexamethasone treatment, the mean concentration was, nevertheless, significantly lower ($P < 0.01$) than on the control night (Table 2.8). All other mean steroid concentrations in each of the three women were significantly lower ($P < 0.001$) on the treatment night than on the control night (Table 2.8). As shown in Figure 2.27, the treatment with dexamethasone for one week suppressed the mean concentrations of all steroid hormones in this group of three women to levels near the detection limits of the assays. Although there was no significant difference in the mean hormone concentrations after the dexamethasone treatment between the group of three women treated with dexamethasone for one week, and the group studied after one dose of dexamethasone, the levels of significance were probably affected by the small number of women in the groups.

b) Effects of Infusion of Tetracosactrin

The profiles of the three women (Subject Nos. 20, 26 and 6) who were infused tetracosactrin after the two doses of dexamethasone are shown in Figures 2.20-2.22. Mean steroid concentrations during the last four hours of the infusion are given in Table 2.7. During the infusions, plasma cortisol and androstenedione concentrations rose to levels that were significantly higher ($P < 0.001$) than occurred during the early morning surge in the control sampling period but, unlike cortisol, plasma androstenedione concentrations did not always plateau and even sometimes showed marked fluctuations. Plasma testosterone concentrations increased during the infusions although only in one subject (No. 6, Figure 2.22) was the mean concentration significantly higher than on the control morning. Plasma oestrone concentrations in two of the subjects (Nos. 6 and 20, Figures 2.22

Figure 2.27. Mean ± SEM concentrations of cortsiol (F), androstenedione (A), testosterone (T), oestrone (E_1) and oestradiol in nine blood samples obtained between 0430 and 0830 hours from three women before (Pre) and after (Post) treatment with dexamethasone 1.5 mg daily for seven days. Levels of significance of differences before and after the dexamethasone treatment are denoted * $P < 0.05$ and *** $P < 0.001$.


µg/dl
15
7
1
Pre Post
F
ng/dl
80
50
20
Pre Post
A
ng/dl
30
20
10
Pre Post
T
ng/dl
5
1
Pre Post
E1
ng/dl
3
1
Pre Post
E2

and 2.20) increased during the infusion, but in the other subject there was no alteration in the levels except for a surge in the first hour of the infusion. Plasma oestradiol concentrations tended to increase slightly, but not significantly, during the infusions in all these women.

The profiles of the two women (Subject Nos. 19 and 7) who were infused tetracosactrin after one week of treatment with dexamethasone are shown in Figure 2.23 and 2.24 respectively. The increases in plasma cortisol and androstenedione concentrations during the infusions were similar, but not as marked or as varied as occurred in the other three women described above and who were studied after two doses of dexamethasone. The increases in the plasma testosterone concentrations during the infusions were not significant. Plasma oestrone and oestradiol concentrations in Subject No. 19 (Figure 2.23) rose significantly ($P < 0.001$) during the infusion whereas the concentrations in the other subject did not rise significantly.

After the infusions, plasma cortisol and androstenedione concentrations fell to near their pre-infusion levels in all four women in whom the sampling period was continued. Testosterone concentrations showed a slight fall towards the end of the infusions but oestrone and oestradiol were maintained at the levels that they had reached during the infusions, even in the woman studied after one week of treatment with dexamethasone.

DISCUSSION

i) Cortisol and Androstenedione

In this study, only plasma cortisol and androstenedione concentrations showed synchronous fluctuations and a diurnal rhythm on the

control night and then were always suppressed by treatment with dexamethasone and increased by infusions of tetracosactrin. It may therefore be concluded that in postmenopausal and ovariectomised women, ACTH directly regulates the acute secretion of only cortisol and androstenedione by the adrenal cortex. The regulation of the adrenal secretion of cortisol by ACTH is well known, but the circadian relationship of plasma cortisol and androstenedione was first demonstrated only recently (Tunbridge et al., 1973). Vermeulen (1976) and Campbell et al. (1976) have subsequently confirmed that the pattern of androstenedione concentrations in postmenopausal women showed the diurnal rhythm that is characteristic of ACTH secretion (Berson and Yalow, 1968). Plasma androstenedione concentrations in postmenopausal women have previously been shown to also be significantly suppressed by dexamethasone treatment and increased by tetracosactrin (Mavoulis and Abraham, 1976; Vermeulen, 1976).

However, the results of some studies have not always supported the conclusion that ACTH regulates the adrenal secretion of androstenedione. Baird et al. (1969) found that the intravenous administration of ACTH increased the concentration of androstenedione in only two of the three subjects. Nevertheless, other findings of Baird et al. (1969) and also of Saez et al. (1972) of very high androstenedione concentrations in the adrenal vein when compared with the concentrations in a peripheral vein suggests that the adrenal cortex secretes androstenedione. Although Greenblatt et al. (1976) found that the adrenal vein concentration of androstenedione did not increase significantly 30 minutes after the intravenous administration of ACTH, even before the injection, the androstenedione concentration in the adrenal vein was not even double the peripheral vein

concentration. Borkowski et al. (1977) found in postmenopausal and ovariectomised women treated with dexamethasone that although cortisol was always undetectable, the plasma concentrations of androstenedione were never less than 50 ng/dl. The reasons for the discrepancies in the results of these studies are at present unclear.

Because this study showed that androstenedione concentrations fluctuated markedly and with a diurnal rhythm, the accurate estimation of the rate of secretion of androstenedione by postmenopausal women requires the determination of androstenedione concentrations in several blood samples obtained at intervals throughout the day. Furthermore conclusions such as that by Marshall et al. (1977), who related the androstenedione concentration in a single blood sample obtained about 0900 hours to plasma oestrone concentrations and to osteoporosis, have limited value. Moreover in the present study, the androstenedione concentration determined in a blood sample obtained at 0900 hours did not correlate with the mean 24 hour androstenedione concentration or with the oestrone concentration also determined in the 0900 hour blood sample. (Appendix IV).

The occasional asynchrony of the plasma cortisol and androstenedione concentrations that was apparent in some of the women in this study, particularly during the tetracosactrin infusions, had also been observed in the intensive hormone profile studies (Parts 2 a and 2 b). These asynchronous fluctuations may result from different cellular responses within the adrenal to ACTH. However, and as discussed in Part 2 a, they were more probably caused by alterations in hepatic blood flow which affected only the plasma concentration

of androstenedione and not of cortisol whose clearance is entirely extrasplanchnic (Baird et al., 1969). However this phenomenon requires further investigation.

The reduced steroid responses to ACTH in the women studied after one week of dexamethasone, when compared with the responses in the other women, presumably resulted from hypotrophy of the adrenal cortex. However, the androstenedione and cortisol response to ACTH in the women varied, perhaps because of different rates of clearance of these steroid in the women. ACTH and glucocorticoids have themselves been found to affect the clearance rates of some steroids, including cortisol (Bardin et al., 1968; Kusama et al., 1970; de Lacerda et al., 1973; Messerli et al., 1976; Nishida et al., 1977). However, further studies are necessary to determine the exact factors regulating the plasma androstenedione concentration especially because androstenedione, unlike cortisol, does not apparently regulate its own secretion by a negative feedback axis.

ii) Testosterone, Oestrone and Oestradiol

The production of plasma testosterone, oestrone and oestradiol after the menopause is not directly regulated by ACTH because in this study the plasma concentrations did not show fluctations which were synchronous with those of cortisol and androstenedione, were not suppressed by a single dose of dexamethasone and were not always increased by infusions of tetracosactrin. These findings contrast with those of Campbell et al. (1976) who detected a diurnal rhythm in plasma testosterone and oestrone concentrations, and of Vermeulen (1976) who found such a rhythm for oestrone (but not testosterone or oestradiol) in postmenopausal women. However in these two studies blood samples were only obtained at intervals of two to four hours

and, furthermore, fluctuations in the plasma concentrations of these steroids were not always synchronous with those of androstenedione.

Previous adrenal suppression studies of postmenopausal and ovariectomised women by Saez et al. (1972), Maroulis and Abraham (1976), Vermeulen (1976) and Borkowski et al. (1977) have, generally shown effects of plasma testosterone, oestrone and oestradiol that were similar to those found in this study, and in the previous study (Part 2 b). Because plasma concentrations of testosterone, oestrone and oestradiol were usually unmeasurable after one week of treatment with dexamethasone, and increased with the administration of tetracosactrin, it may be concluded that ACTH does regulate, albeit not directly, the production from the adrenal of plasma testosterone, oestrone and oestradiol in postmenopausal and ovariectomised women.

In the adrenal vein catheterisation studies by Baird et al. (1969) and Saez et al. (1972), the amounts of testosterone, oestrone and oestradiol secreted by the adrenal was almost insignificant in comparison to the amount of androstenedione secreted. In this study the plasma androstenedione and cortisol concentrations in the four hours after the completion of the tetracosactrin infusion fell to their pre-infusion levels, but plasma testosterone, oestrone and oestradiol levels remained elevated. Thus, this plasma testosterone oestrone and oestradiol were probably not produced by direct adrenal secretion, and were probably derived from extraglandular sources. Isotopic studies have not only clearly demonstrated the significance of peripheral production from androstenedione of testosterone and oestrone, either of which may then be converted to plasma oestradiol (Longcope et al., 1969; Olivo et al., 1973) but they have also

suggested that all plasma oestrone after the menopause may be derived from the peripheral aromatisation of androstenedione (Grodin et al., 1973). The role of the periphery in the production of these steroids is presently unclear. However, it is possible that virtually all testosterone, oestrone and oestradiol after the menopause is produced only from adrenal androstenedione in the periphery which then 'stores' these steroids or, alternatively, releases them into the circulation. In this study, the continued production of plasma testosterone, oestrone and oestradiol after the tetracosactrin infusion had ceased, may have resulted from the release from extraglandular sources ('stores') which had become depleted during the week of treatment with dexamethasone, but repleted, at least partially, by the peripheral conversion of androstenedione secreted by the adrenal during the infusion. Because the number of subjects in this study was small, further studies are obviously required to determine whether the only adrenal contribution to plasma steroids after the menopause is the secretion of androstenedione, and furthermore, to determine the exact role of the periphery in the production of plasma oestrogens and their androgenic prehormones after the menopause.

Part 2 d) EXTRAGLANDULAR PRODUCTION OF PLASMA OESTROGENS

As described in detail in Part 1, significant peripheral conversion of androstenedione and testosterone to oestrone and oestradiol respectively and interconversion of oestrone and oestradiol have been demonstrated by Longcope et al. (1968 & 1969). Other isotopic studies (MacDonald et al., 1967; Siiteri and MacDonald, 1973; Grodin et al., 1973; Bates, 1978; MacDonald et al., 1978) have concluded that all oestrone in postmenopausal women could be derived from the extraglandular conversion of androstenedione, and that the degree of this aromatisation is directly related to body weight. Furthermore, in vitro studies (Schindler et al., 1972; Nimrod and Ryan, 1975) have demonstrated that human fat tissue has the ability to aromatise androstenedione to oestrone. Thus, adipose tissue has come to be regarded as the predominant site of the peripheral production of oestrogens after the menopause. Recent isotopic studies by Southren et al. (1974) and Gordon et al. (1975) showed that subjects with cirrhosis or hyperthyroidism had increased peripheral aromatisation of androgens to oestrogens.

One aim of this study was to explore further the extraglandular production of oestrogens after the menopause (and particularly the regulatory factors) by examining the interrelationships of the plasma concentrations of precursors and products. This was carried out by infusing unlabelled steroid hormones and examining the products, and by determining the effects of body weight and hyperthyroidism on plasma steroid concentrations in postmenopausal and ovariectomised women.

The results of the ACTH suppression and stimulation studies

(Part 2 c) suggested that oestrogens may also be 'stored' in the periphery. This possibility was explored in this study by comparing mean plasma oestrone, oestradiol and testosterone concentrations in normal postmenopausal and ovariectomised women with those in women in whom adrenal secretion of steroids had been suppressed by dexamethasone for one day or for one week. The possible existence of peripheral 'stores' was also explored by examining the changes in the plasma steroid concentrations after the infusion of unlabelled steroids or ACTH in women in whom the 'stores' had previously been depleted by dexamethasone treatment for one week.

Throughout this study it has been assumed that there was no significant contribution to steroid production by the postmenopausal ovary and that there was no adrenal secretion of testosterone, oestrone or oestradiol. It is realised however that although such assumptions are based largely on earlier studies (Parts 2 b & 2 c), clarification by further studies is awaited.

METHOD

i) Correlations of Precursor-Product Concentrations

The conversions and interconversions of androstenedione, testosterone, oestrone and oestradiol (Figure 1.4.) were studied by examining the correlations between plasma concentrations in 28 women - the 16 postmenopausal women whose results were described in Part 2 a (Subject Nos. 1-16), the 10 ovariectomised women described in Part 2 b(Subject Nos. 17-26) and two other women (Subject Nos. 36 & 39) who were 31% and 73% above their ideal body weight (Geigy, 1970). Clinical details of all these

subjects are given in Table 2.4. The mean ± SD age and weight of this group of 28 women were 50.9 ± 4.2 years and 63.9 ± 10.7 Kg. Blood samples were obtained from each woman every 20-30 minutes for 4-24 hours for the determination of the plasma oestrone, oestradiol and testosterone concentrations. The plasma androstenedione concentrations were also determined in the 11 women from whom blood samples were obtained for 24 hours.

ii) Infusions of Unlabelled Steroids

Androstenedione: Two ovariectomised women (Subject Nos. 22 & 23) were studied without prior treatment with dexamethasone. Blood samples were obtained every 30 minutes for 18-24 hours and, during the afternoon, (when ACTH secretion was expected to be low), androstenedione was infused into a forearm vein at 250 µg/hour for six hours after which sampling was continued for a further six hours.

Three other women (Subject Nos. 27-29) were also infused androstenedione but after prior treatment with dexamethasone (1.5 mg daily) for one week to suppress adrenal secretion of androstenedione and deplete peripheral sources of plasma androgens and oestrogens. In one woman (Subject No. 27) cortisol was included in the mixture and infused at 2.5 mg/hr to determine whether pump malfunction could account for fluctuations in the plasma androstenedione concentrations. During the infusion of this mixture, the woman's posture was kept supine and food, but not fluids, was witheld so that the major alterations in hepatic blood flow that occur with alterations in posture and ingestion of food could not occur. Blood samples were obtained from all

three subjects every 30 minutes for 18-24 hours commencing at 2400 hours. Plasma cortisol, androstenedione, testosterone, oestrone and oestradiol were determined in all blood samples from these five women.

Testosterone: One postmenopausal woman (Subject No. 30) and two ovariectomised women (Subject Nos. 29 and 31) were studied after dexamethasone treatment (1.5 mg daily) for one week. Blood samples were obtained every 30 minutes for 16-24 hours. Four to nine hours after sampling commenced, testosterone was infused at 45 µg/hour for six hours after which sampling was continued for a further 6-12 hours. Plasma cortisol, androstenedione, testosterone, oestrone and oestradiol concentrations were determined in all the blood samples.

Oestrone and Oestradiol: One postmenopausal woman and two ovariectomised women were studied after dexamethasone treatment for one week. Blood samples were obtained every 30 minutes for 14-18 hours. Four to six hours after sampling was commenced, oestrone was infused at 20 µg/hr in one woman (Subject No.34) and oestradiol was infused at 10 µg/hr in the two other women (Subject Nos. 32 and 33). After the completion of the six hour infusions, sampling was continued for 4-6 hours. Plasma oestrone and oestradiol were determined in all the blood samples and, in addition, androstenedione, cortisol and testosterone levels were determined in the pre-infusion samples.

iii) Effects of Body Weight

One obese 56 year old postmenopausal woman (Subject No. 39) who weighed 107.5 Kg and was 73% above the ideal weight for her height was studies by obtaining blood samples every 30 minutes

for 24 hours. The plasma cortisol, androstenedione, testosterone, oestrone and oestradiol concentrations determined in these blood samples were compared with those from normal postmenopausal women who were within 12% of the ideal weight for their height (Part 2 a).

The relation of body weight and plasma testosterone, oestrone and oestradiol concentrations was also examined in the group of 28 women described above. In the 11 women in this group in whom blood sampling was performed for 24 hours, body weight was also compared with the androstenedione concentration.

iv) Effects of Thyroid Hormones

A 56 year old woman with hyperthyroidism due to thyroxine overdosage, was studied. About fifteen years prior to the study hypothyroidism had been diagnosed (at another hospital) and the woman commenced treatment with thyroxine (0.3 mg) each morning. Five years prior to the study, a hysterectomy and bilateral salpingoophorectomy was performed because of menorrhagia. Histology of the endometrium showed adenomatous hyperplasia. The woman had been referred to the Menopuase Clinic for treatment of 'flushes' she had had since the pelvic clearance operation. However, because her plasma thyroxine concentration was 249 nMol/L (upper limit of normal range = 160 nMol/L) and the free thyroxine index was 244, hyperthyroidism was diagnosed. On the days of the study, blood samples were obtained every 30 minutes for 12 hours beginning at 2200 hours. The woman was studied on a control night and then on the following night when dexamethasone (2 mg) was prescribed at 2400 hours. Plasma cortisol, androstenedione,

testosterone, oestrone and oestradiol concentrations were determined in all the blood samples. After the study, the plasma thyroxine dosage was halved, and three months later, the woman was clinically euthyroid although the plasma thyroxine was still slightly elevated at 172 nMol/L and the free thyroxine index was 164 (normal range 90-170). Readmission for a further profile study was not then possible.

v) Peripheral 'Stores'

The mean plasma testosterone, oestrone and oestradiol concentrations in the 26 postmenopausal or ovariectomised women discussed in Parts 2 a and 2 b, were regarded as the normal concentrations for the comparative purposes of this study, as were the plasma cortisol and androstenedione concentrations determined between 0430 and 0830 hours in the eight women (Subject Nos. 6,20,26,16,25,7,19 & 21) studied in Part 2 c. The control plasma steroid concentrations after a single dose of dexamethasone were those in the five women (Subject Nos. 6,20, 26,16 & 25) from whom blood samples were obtained between 0430 and 0830 hours as described in Part 2 c. The plasma steroid concentrations after dexamethasone treatment for one week were those in the group of 11 women (Subject Nos. 7,19,21, 27 -34) from whom blood samples were obtained for 4-14 hours as described in Part 2 b. The possibility of peripheral sources of plasma oestrone, oestradiol or testosterone was explored by comparing the mean steroid concentrations in these three groups of women and assuming that the oestrone, oestradiol and testosterone circulating after acute suppression of the adrenal by a single

dose of dexamethasone resulted from peripheral sources and that these 'stores' became depleted by treatment with the dexamethasone for one week.

The possible role of the periphery as a 'store' of plasma steroids after the menopause was also studied by examining plasma levels after circulating unconjugated steroids produced during steroid or ACTH infusions would have been expected to have cleared from the circulation. In this study, steroids whose levels were raised more than two hours after infusion of ACTH or steroids were regarded as being produced from 'stores' which had previously been depleted by dexamethasone treatment but were repleted during the infusion. The mean pre-infusion oestrone, oestradiol and testosterone concentrations after dexamethasone treatment for one week were compared with the mean concentrations in two four or five hour sampling periods that commenced two or four hours after the completion of infusion of ACTH (Subject Nos. 7 and 19, Part 2 c), androstenedione (Nos. 21,27 & 28), testosterone (Nos. 29,30 & 31) oestradiol (Nos. 32 & 33) or oestrone (No. 34).

RESULTS

i) Correlations of Precursor-Product Concentrations

The mean plasma steroid concentrations of each of the 28 women in this study are shown in Table 2.4. The mean $\pm$ SD androstenedione, testosterone, oestrone and oestradiol concentrations in the group of women were 74.2 $\pm$ 22.2 ng/dl (n = 11), 35.8 $\pm$ 13.0 ng/dl (n = 28), 5.2 $\pm$ 1.8 ng/dl (n = 28) and 3.2 $\pm$ 1.1 ng/dl (n = 28) respectively. The correlation

coefficients between the mean plasma androstenedione, testosterone, oestrone and oestradiol concentrations in the women are shown in Figure 2.28. The correlation between plasma androstenedione and testosterone was significant ($P < 0.05$) - Figure 2.29. - but all other correlations including that between androstenedione and oestrone (Figure 2.30.) were not significant.

ii) Infusions of Unlabelled Steroids

Androstenedione: The hormone profiles of the two ovariectomised women (Subject Nos. 22 and 23) infused androstenedione without prior treatment with dexamethasone are shown in Figures 2.31. and 2.32. respectively, and their mean steroid concentrations before the infusion, during the last four hours of the infusion and in the second to sixth hours after the infusion are shown in Table 2.9. Before the infusions commenced, the plasma concentrations and fluctuations of all steroid hormones were similar to those previously described for postmenopausal and ovariectomised women (Parts 2 a and 2 b) and, in particular, there was the usual increase in cortisol and androstenedione concentrations in the early morning. During the androstenedione infusions, plasma androstenedione rose to concentrations near the upper limit of the physiological range in both subjects but never reached a plateau. Meanwhile, the plasma levels of cortisol were similar to those normally seen in the afternoon. In both women, plasma testosterone and oestrone (but not oestradiol) concentrations rose during the infusion to levels that, in the last four hours, were significantly higher than pre-infusion levels ($P < 0.001$). Furthermore they remained significantly elevated

Figure 2.28. Correlation coefficients (r) between mean plasma androstenedione, testoserone, oestrone and oestradiol concentrations in postmenopausal or ovariectomised women.

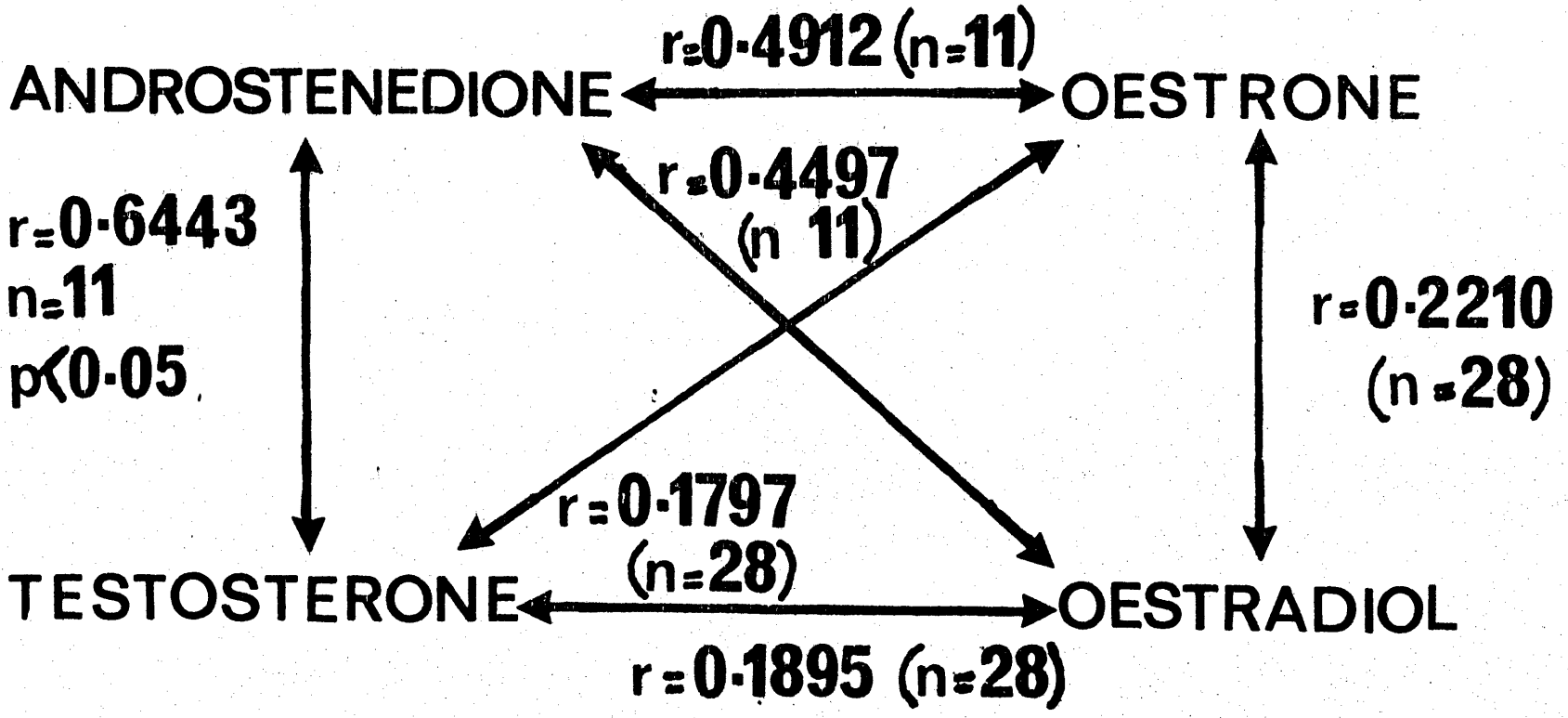

r=0·4912 (n=11)
ANDROSTENEDIONE
OESTRONE
r=0·4497
(n 11)
r=0·6443
n=11
p<0·05
r=0·2210
(n=28)
r=0·1797
(n=28)
TESTOSTERONE
OESTRADIOL
r=0·1895 (n=28)

Figure 2.29. Correlation of mean (24 hour) plasma androstenedione concentration and mean testosterone concentration in 11 postmenopausal or ovariectomised women.

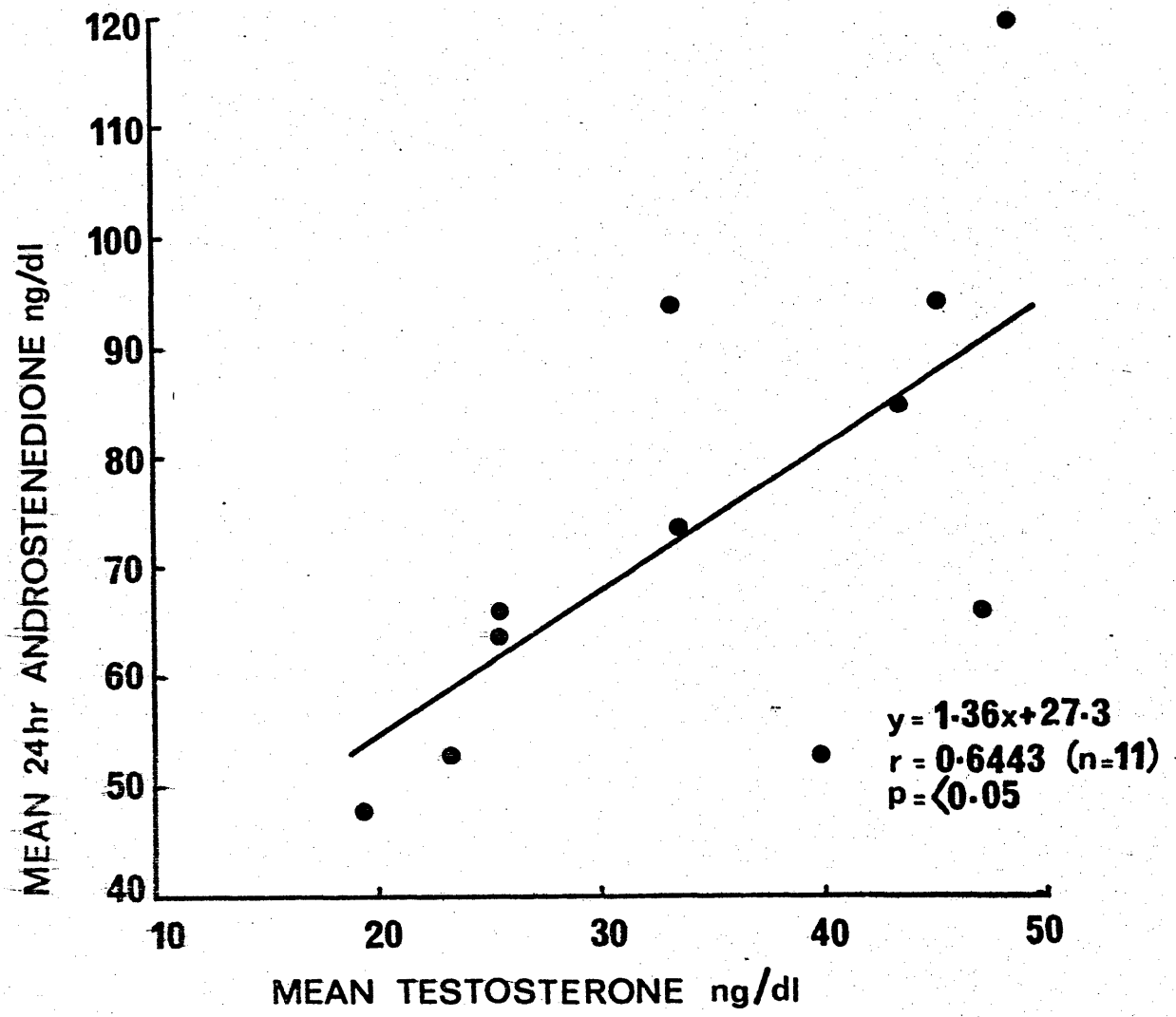

120
110
100
90
80
70
60
50
40
MEAN 24hr ANDROSTENEDIONE ng/dl
10
20
30
40
50
MEAN TESTOSTERONE ng/dl
y = 1·36x+27·3
r = 0·6443 (n=11)
p = <0·05

Figure 2.30. Correlation of mean (24 hour) plasma androstenedione concentration and mean oestrone concentration in 11 postmenopausal or ovariectomised women.

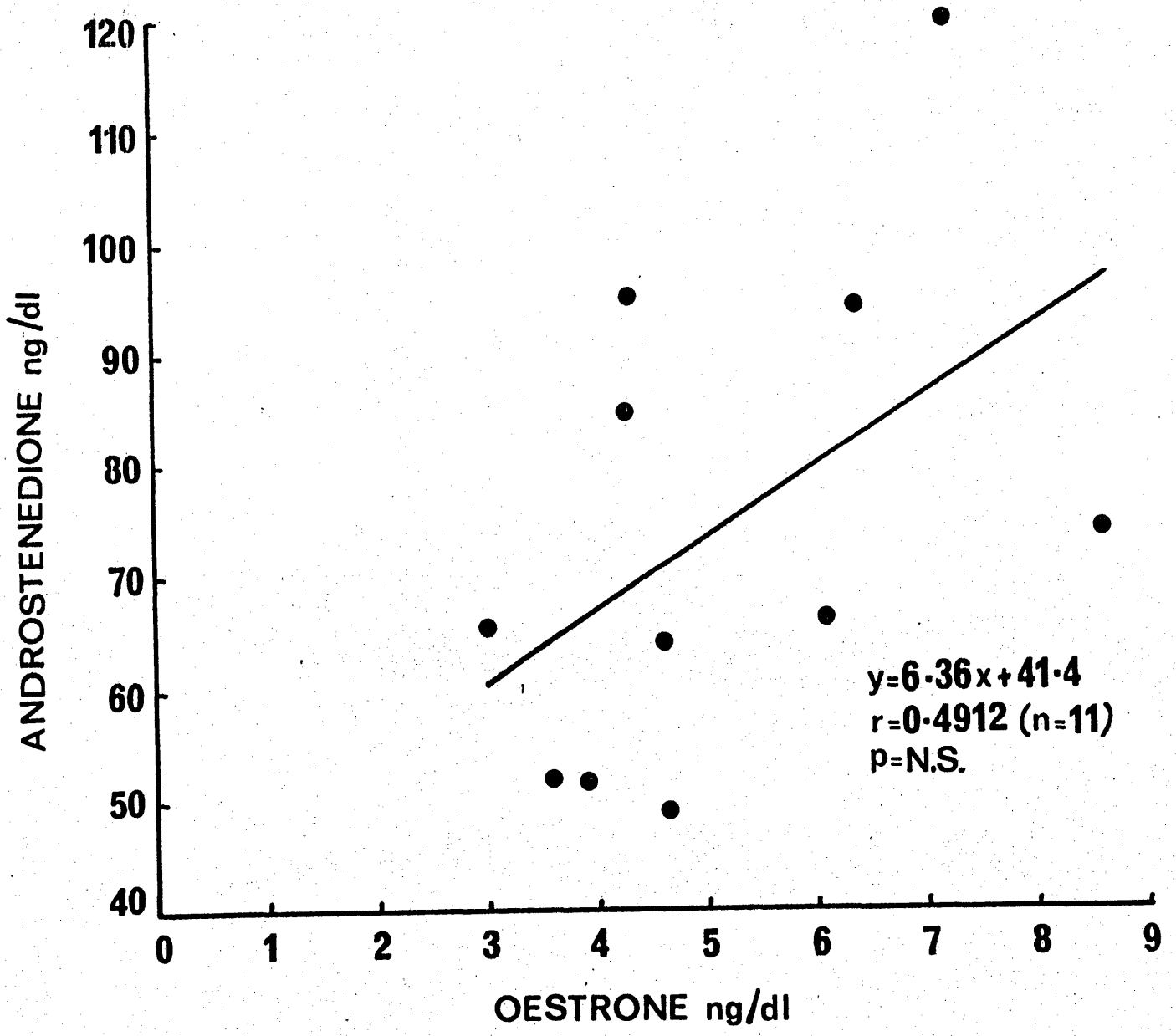

ANDROSTENEDIONE ng/dl
120
110
100
90
80
70
60
50
40
0
1
2
3
4
5
6
7
8
9
OESTRONE ng/dl
y=6·36x+41·4
r=0·4912 (n=11)
p=N.S.

Figure 2.31. Plasma steroid concentrations in blood samples obtained every 30 minutes from an ovariectomised woman (Subject No. 22), who was infused androstenedione 250 µg/hour for six hours during the afternoon.

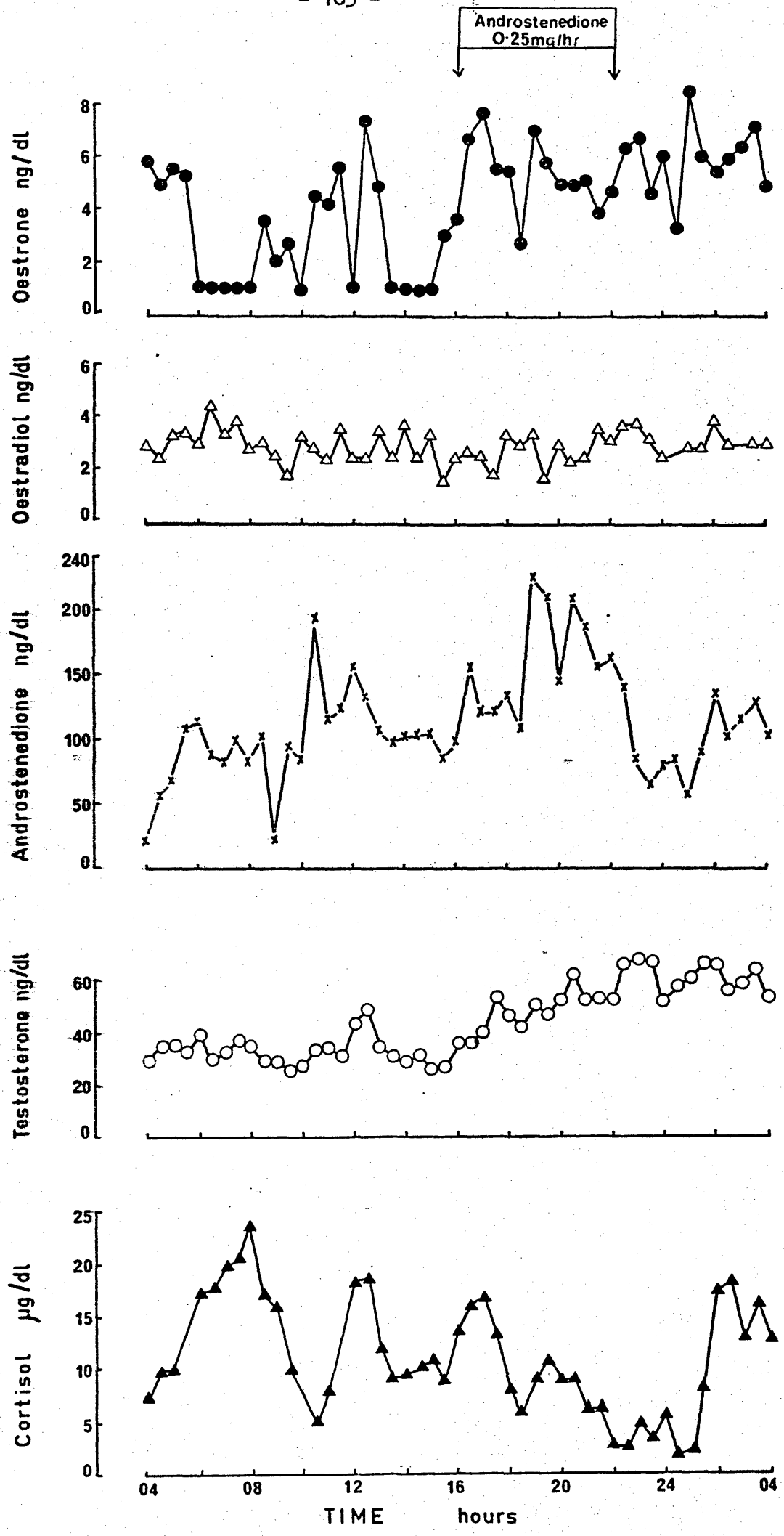

Androstenedione
0·25mg/hr
Oestrone ng/dl
0
2
4
6
8
Oestradiol ng/dl
0
2
4
6
Androstenedione ng/dl
0
50
100
150
200
240
Testosterone ng/dl
0
20
40
60
Cortisol µg/dl
0
5
10
15
20
25
04
08
12
16
20
24
04
TIME hours

Figure 2.32. Plasma steroid concentrations in blood samples obtained every 30 minutes from an ovariectomised woman (Subject No. 23) who was infused androstenedione 250 µg/hour for six hours during the afternoon.

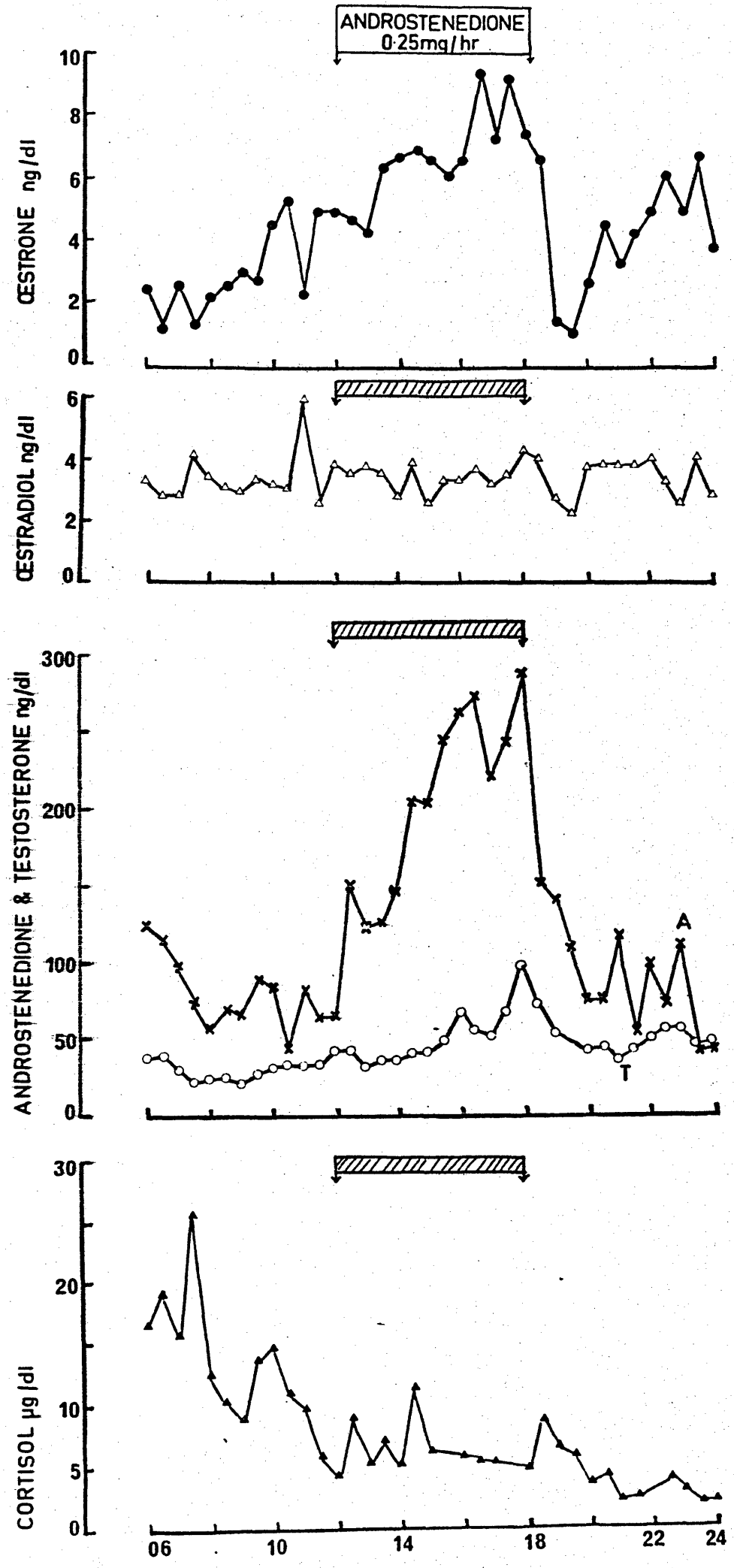

ANDROSTENEDIONE
0·25mg/hr
ŒSTRONE ng/dl
10
8
6
4
2
0
ŒSTRADIOL ng/dl
6
4
2
0
ANDROSTENEDIONE & TESTOSTERONE ng/dl
300
200
100
50
0
A
T
CORTISOL µg/dl
30
20
10
5
0
06
10
14
18
22
24

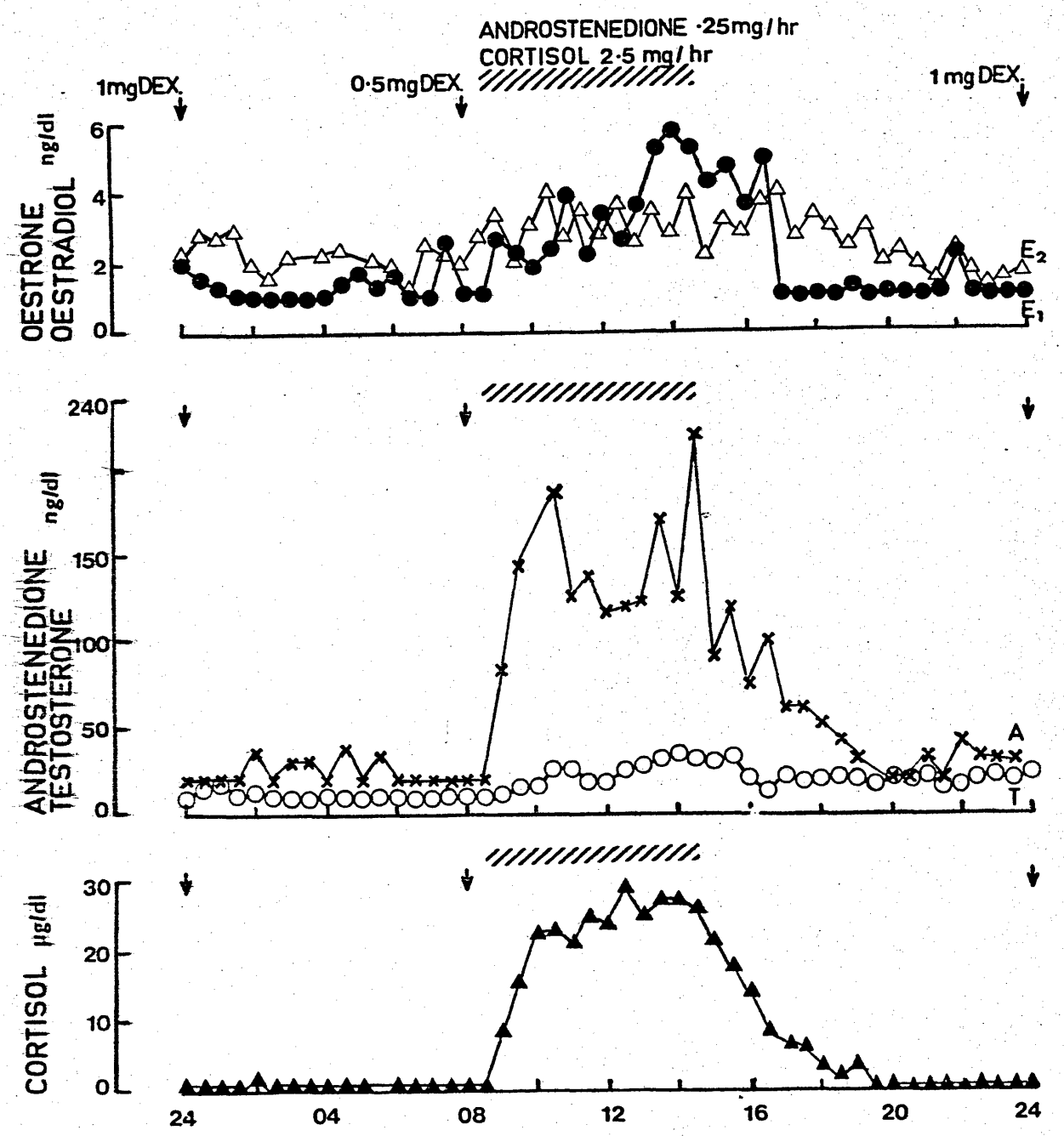
Table 2.9. Mean ± SD steroid concentrations in blood samples obtained every 30 minutes from two ovariectomised women infused androstenedione at 250 ug/hr for six hours without prior treatment with dexamethasone before (Pre) and during the last four hours of the infusion and in a four hour period commencing two hours after the completion of the infusion (Post). (Significance of difference from pre-infusion levels: *** p = < 0.001, ** p = <0.01, * p = <0.05. (M) denotes the Mann Whitney U test. N. the number of samples.

NO	SAMPLING PERIOD (h)	N	F ug/dl	A ng/dl	T ng/dl	E_1 ng/dl	E_2 ng/dl
22	Pre (0400-1400)	25	13.8 ± 5.4	97.3 ± 35.5	32.9 ± 5.2	3.1 ± 2.1	2.9 ± 0.6
	During (1800-2200)	9	7.5 ± 2.4***(M)	167.1 ± 41.7***	51.5 ± 5.7***	5.1 ± 1.2*(M)	3.1 ± 0.5
	Post (2400-0400)	9	10.8 ± 6.4	97.4 ± 24.9	59.7 ± 5.6***	6.1 ± 1.4***	3.1 ± 0.4
23	Pre (0600-1200)	13	12.8 ± 5.6	79.2 ± 24.7	31.2 ± 6.7	3.0 ± 1.4	3.4 ± 0.9
	During (1400-1800)	9	6.0 ± 2.4**(M)	230.1 ± 44.1***(M)	55.3 ± 18.6***(M)	7.3 ± 1.2	3.4 ± 0.5
	Post (2000-2400)	9	3.0 ± 0.9***(M)	74.2 ± 27.4	47.1 ± 6.8***	4.6 ± 1.3	3.6 ± 0.5

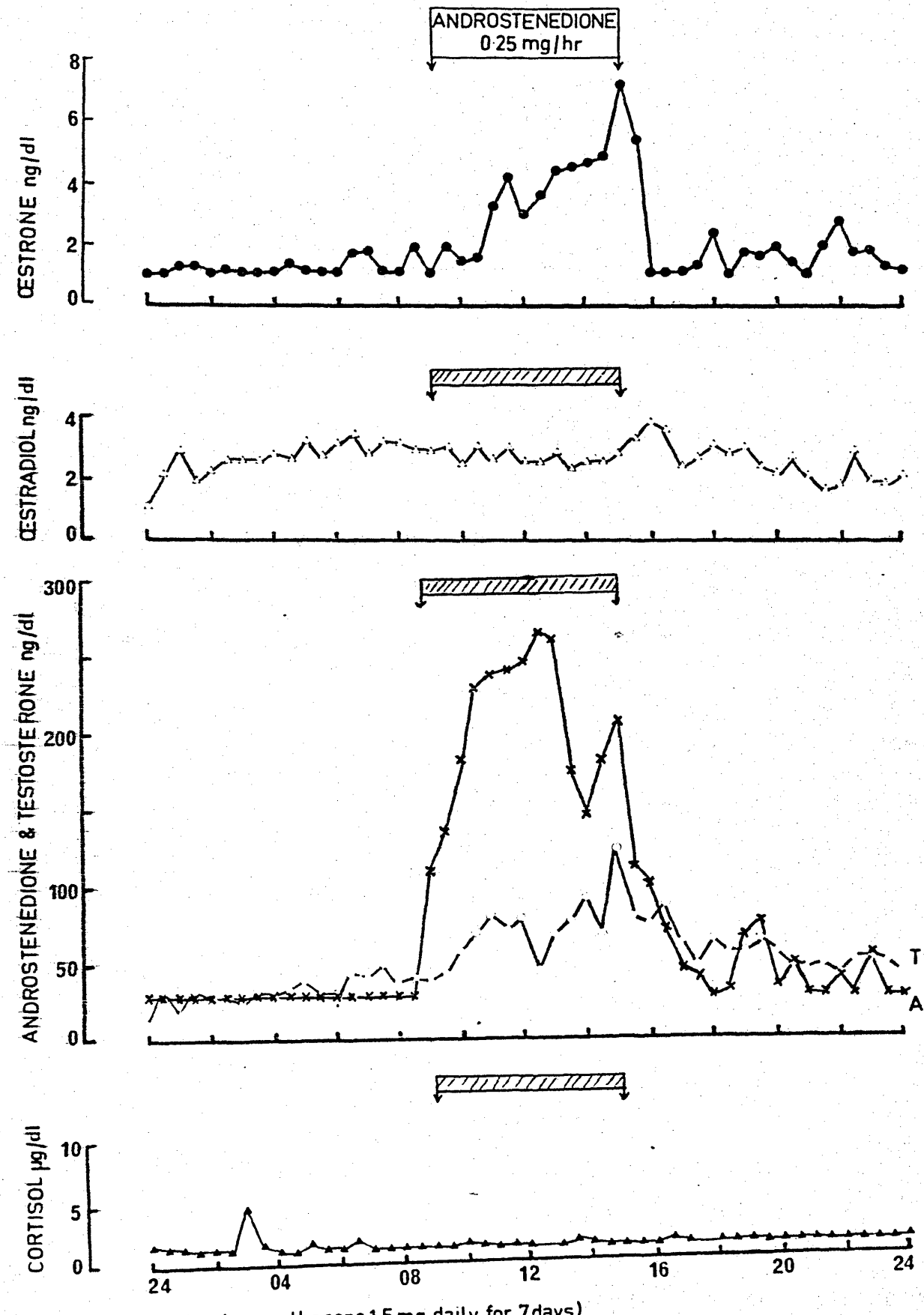
after the completion of the infusion (Table 2.9.) while the concentration of androstenedione fell back to pre-infusion levels.

The hormone profiles of the three women (Subject Nos. 21, 27 and 28) who were infused androstenedione after prior treatment with dexamethasone for one week are shown in Figures 2.25., 2.33. and 2.34. respectively. The mean steroid concentrations before and during the last four hours of the infusion are shown in Table 2.10. This last table also shows the steroid concentrations for Subject No. 21 during the second to fourth hours after the infusion and for the other two subjects (Nos. 27 & 28) during the fourth to ninth hours after which the sampling was discontinued. Prior to the infusion, the plasma steroids were almost all undetectable except for oestradiol in Subject No. 28 as previously discussed in Part 2 b. During all infusions plasma androstenedione levels increased markedly to near the upper limit of the physiological range, but did not reach a plateau, even in Subject No. 27 (Figure 2.33.) who was infused both cortisol and androstenedione. In this subject, the cortisol levels rose to a smooth plateau of about 25 µg/dl. Testosterone and oestrone levels also rose during the infusions but oestrone levels showed fluctuations similar to those seen in normal postmenopausal women. After the infusions, plasma androstenedione concentrations always fell to their pre-infusion values, but the falls in testosterone and oestrone were variable such that in one subject (No. 28, Figure 2.34.) the plasma testosterone value was still significantly higher ($P < 0.001$) than the pre-infusion level long after the infusion had ceased. During the infusion, the plasma

Figure 2.33. Plasma steroid concentrations in blood samples obtained every 30 minutes from an ovariectomised woman (Subject No. 27) after treatment with dexamethasone (1.5 mg daily) for one week, and when androstenedione and cortisol were infused together at a rate of 250 µg/hour and 2.5 mg/hour respectively for six hours.


ANDROSTENEDIONE ·25mg/hr
CORTISOL 2·5 mg/hr
1mgDEX
0·5mgDEX
1 mg DEX
OESTRONE
OESTRADIOL ng/dl
6
4
2
0
E2
E1
240
150
100
50
0
ANDROSTENEDIONE
TESTOSTERONE ng/dl
A
T
30
20
10
0
CORTISOL µg/dl
24
04
08
12
16
20
24

Figures 2.34. Plasma steroid concentrations in blood samples obtained from a postmenopausal woman (Subject No. 28) after treatment with dexamethasone (1.5 mg daily) for one week and when androstenedione was infused at a rate of 250 µg/hour for six hours.



(after dexamethasone 1·5 mg daily for 7 days)

Table 2. 10. Mean ± SD plasma steroid concentrations in blood samples obtained every 30 minutes before infusion (Pre), during the last four hours of a six hour infusion of androstenedione (A) or androstenedione plus cortisol (A + F), and after the infusion (Post). All three subjects had had treatment with dexamethasone (1.5mg daily) for one week prior to the study. The figures in brackets denote the hours after the completion of the infusion during which the sampling period occurred . N. denotes the number of samples. (Significance of difference from pre-infusion levels: *** p = <0.001, ** p = <0.01, * p = <0.05. (M).denotes the Mann Whitney U test.

NO	SAMPLING PERIOD	N.	F µg/dl	A ng/dl	T ng/dl	E_1 ng/dl	E_2 ng/dl
	Pre	18	<1.0	<26.6	<10.0	1.6 ± 0.9	1.4 ± 0.4
21	During A	9	<1.0	226.4 ± 45.9***(M)	39.0 ± 6.9***(M)	8.1 ± 1.5	1.0
	Post (2-4)	4	<1.0	39.9 ± 22.4***(M)	27.5 ± 2.1***(M)	5.8 ± 0.9***	1.0
	Pre	18	<1.0	27.6 ± 2.5	10.5 ± 0.7	1.3 ± 0.4	2.1 ± 0.5
27	During A+F	9	25.4 ± 2.4***(M)	148.1 ± 2.4***(M)	26.4 ± 5.6***(M)	3.8 ± 1.3	3.4 ± 0.6***
	Post (4-9)	11	1.3 ± 0.9	32.4 ± 5.7	17.5 ± 2.3	1.1 ± 0.4	2.1 ± 0.6
	Pre	19	1.3 ± 0.8	27.8 ± 3.0	31.4 ± 8.9	1.2 ± 0.3	2.6 ± 0.6
28	During A	9	1.1 ± 0.1	215.8 ± 41.2***(M)	77.2 ± 22.2***(M)	4.3 ± 1.2***(M)	2.7 ± 0.2
	Post (4-9)	11	<1.0	30.5 ± 7.8	49.2 ± 7.0***	1.8 ± 0.7**(M)	2.2 ± 0.5

oestradiol concentration rose only in one subject (No. 27- Figure 2.33.) and did not alter in the other two subjects.

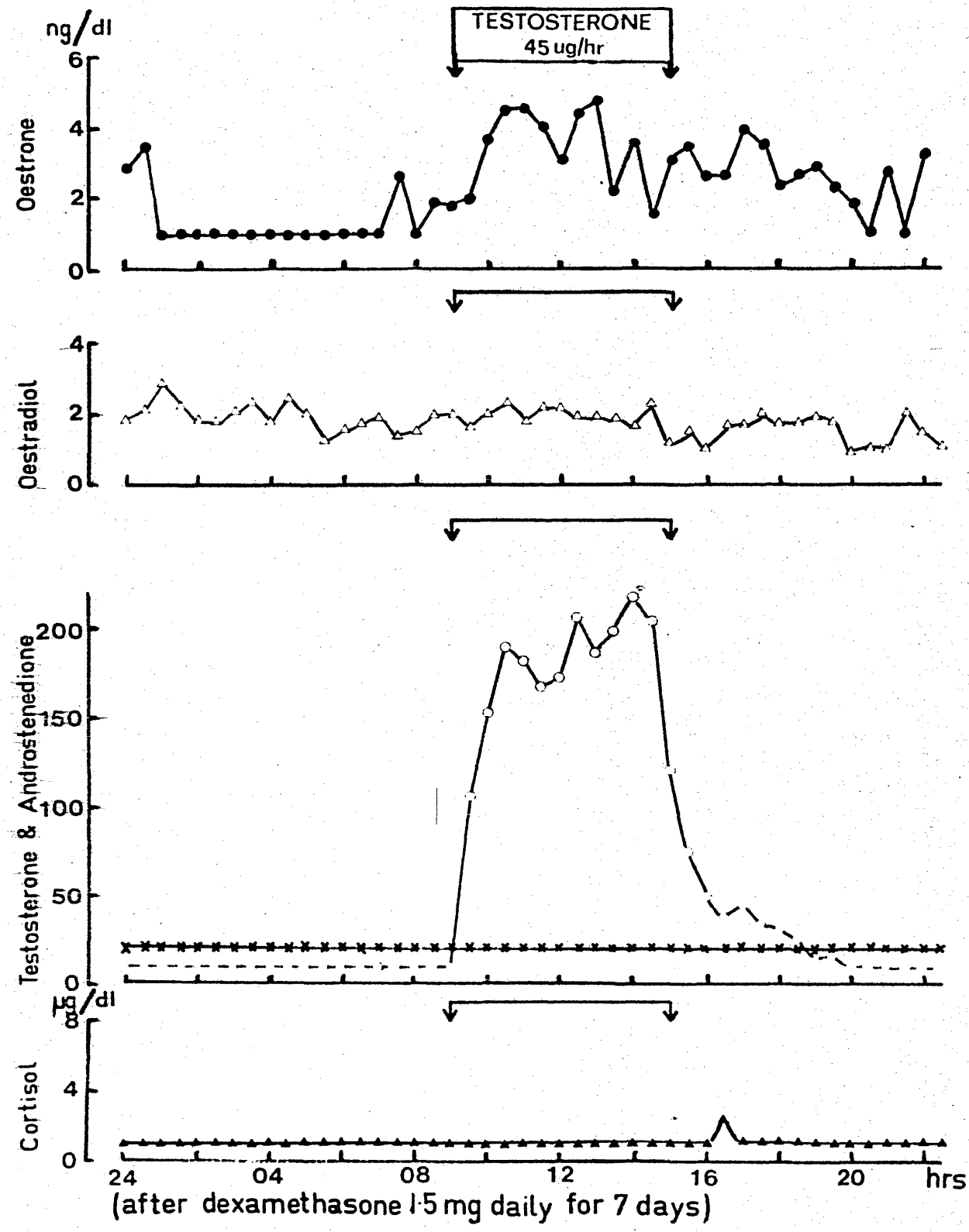
Testosterone: The hormone profiles of Subject Nos. 29,30 & 31 are shown in Figures 2.35., 2.36. & 2.37. respectively, and their mean steroid concentrations prior to the infusion, during the last four hours of the infusion and in a four hour period commencing two or three hours after the completion of the infusion are shown in Table 2.11.

Prior to the infusion, the steroid concentrations were near the limits of detection except for the postmenopausal woman (Subject No. 30) as previously discussed in Part 2 b. During the infusions, plasma testosterone concentrations rose, usually to a plateau about 150-200 ng/dl and marked fluctuations in the testosterone level were uncommon.

In the two ovariectomised women (Subject Nos. 29 & 31), plasma oestrone levels also rose significantly during the infusions but, unlike testosterone, did not fall back to their pre-infusion levels, even 12 hours after the infusion had ceased. Oestradiol levels in these two subjects did not rise during or after the infusions. However, in the postmenopausal woman (Subject No. 30) the plasma oestradiol, but not oestrone, concentrations rose significantly ($P < 0.001$) during the testosterone infusion. After the completion of the infusion, the testosterone and oestradiol levels fell, the latter to the pre-infusion level.

Plasma androstenedione concentrations in two of the three subjects did not alter during the infusions, but in the third subject (No. 31), there was a slight rise towards the end of

Figure 2.35. Plasma steroid concentrations in blood samples obtained every 30 minutes for 24 hours from an ovariectomised woman (Subject No. 29) after treatment with dexamethasone (1.5 mg daily) for one week, and when testosterone was infused at a rate of 45 µg /hour for six hours.



(after dexamethasone 1·5 mg daily for 7 days)

Figure 2.36. Plasma steroid concentrations in blood samples obtained every 30 minutes for 24 hours from a postmenopausal woman (Subject No. 30) after treatment with dexamethasone (1.5 mg daily) for one week, and when testosterone was infused at 45 µg/hour for six hours.

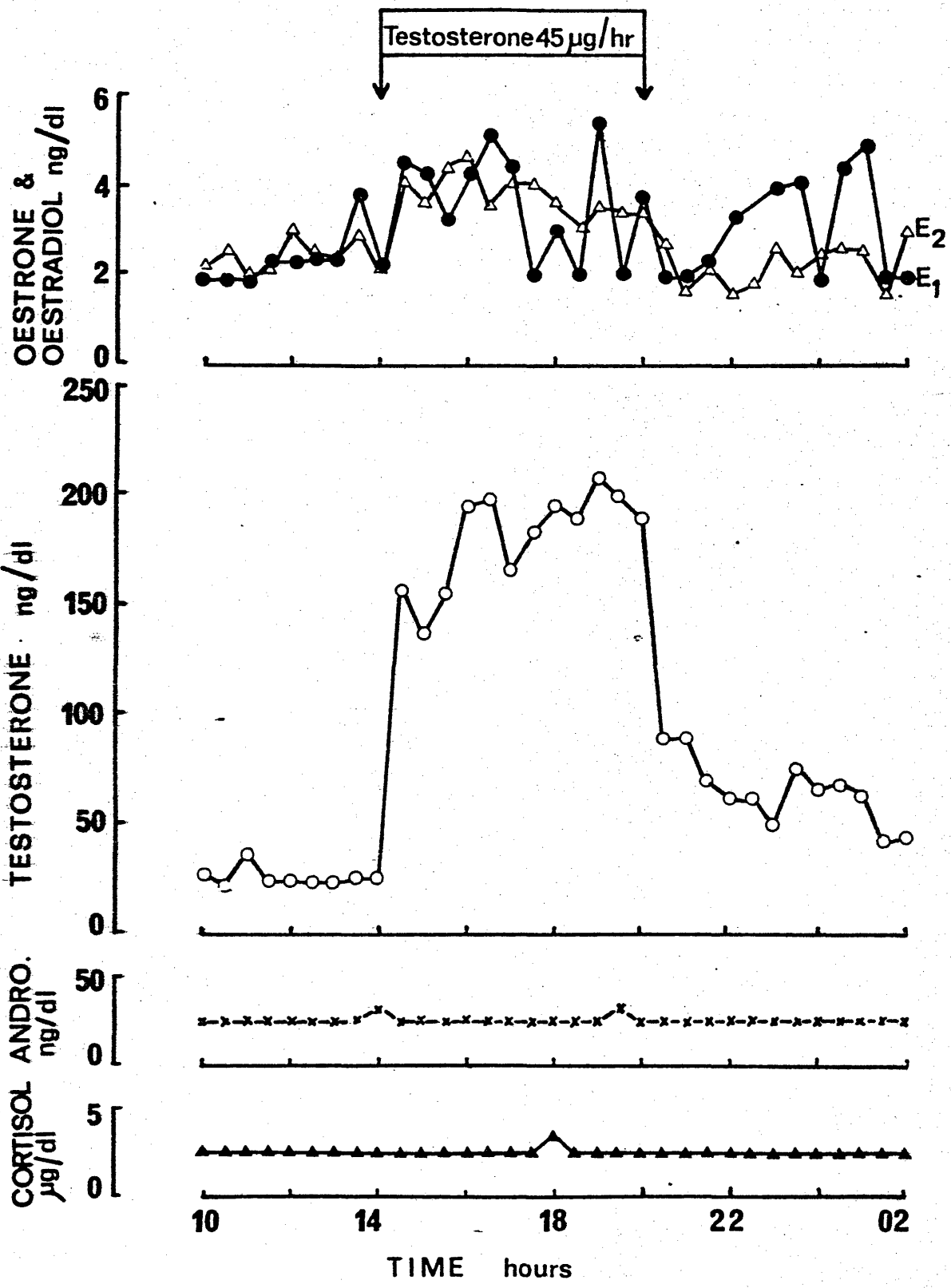

Testosterone 45 µg/hr
OESTRONE & OESTRADIOL ng/dl
6
4
2
0
E2
E1
250
200
150
100
50
0
TESTOSTERONE ng/dl
CORTISOL µg/dl
5
0
ANDRO. ng/dl
50
0
10
14
18
22
02
TIME hours

Figure 2.37. Plasma steroid concentrations in blood samples obtained every 30 minutes for 24 hours from an ovariectomised woman (Subject No. 31) after treatment with dexamethasone (1.5 mg daily) for one week, and when testosterone was in fused at 45 µg/hour for six hours.

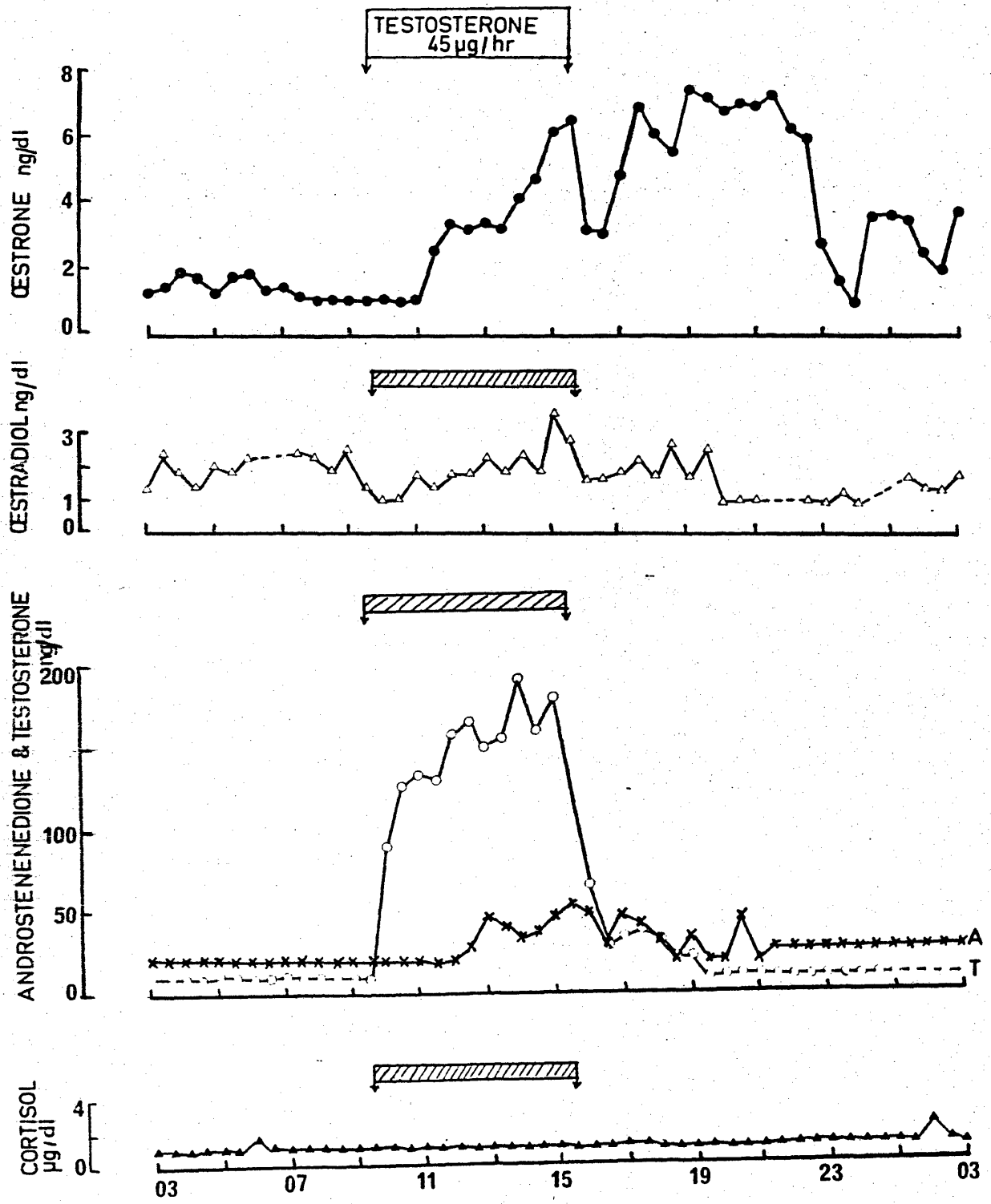

TESTOSTERONE
45 µg/hr
ŒSTRONE ng/dl
8
6
4
2
0
ŒSTRADIOL ng/dl
3
1
0
ANDROSTENENEDIONE & TESTOSTERONE ng/dl
200
100
50
0
A
T
CORTISOL µg/dl
4
0
03
07
11
15
19
23
03

Table 2.11. Mean ± SD steroid concentrations in blood samples obtained every 30 minutes before infusion (Pre), during the last four hours of a six hour infusion of testosterone, and in a four hour period after the infusion (Post). All women had had prior treatment with dexamethasone (1.5mg daily) for one week The figures in brackets denote the hours after the completion ofthe infusion during which the sampling occurred. N. denotes the number of samples. (Significance of difference from pre-infusion levels:*** $p = <0.001$ ** $p = <0.01$, * $p = <0.05$. (M) denotes the Mann Whitney U test.)

NO	SAMPLING VS INFUSION	N.	F µg/dl	A ng/dl	T ng/dl	E_1 ng/dl	E_2 ng/dl
	Pre	19	<1.0	<26.6	<10.0	1.4 ± 0.8	2.0 ± 0.6
29	During	9	<1.0	<26.6	183.9 ± 29.3***(M)	3.5 ± 1.1***	2.0 ± 0.5
	Post (3-7)	9	<1.0	<26.6	15.2 ± 7.3	2.3 ± 0.8**	1.5 ± 0.4
	Pre	9	<1.0	<26.6	28.0 ± 5.3	2.4 ± 0.6	2.5 ± 0.4
30	During	9	<1.0	<26.6	192.5 ± 12.7***(M)	3.6 ± 1.4	3.8 ± 0.5
	Post (2-6)	9	<1.0	<26.6	60.2 ± 12.1***(M)	3.4 ± 1.2	2.4 ± 0.5
	Pre	14	<1.0	<26.6	<10.0	1.3 ± 0.3	1.9 ± 0.4
31	During	9	<1.0	37.6 ± 9.5**(M)	155.9 ± 21.3***(M)	4.1 ± 1.4***(M)	2.2 ± 0.7
	Post (3-7)	9	<1.0	29.9 ± 6.8	12.7 ± 3.6	6.7 ± 0.7***(M)	1.6 ± 0.7

the infusion that was significant ($P < 0.01$) when compared with the pre-infusion value.

Oestradiol and Oestrone: The hormone profiles of the two women (Subject Nos. 32 & 33) who were infused oestradiol are shown in Figures 2.38 and 2.39 respectively. Their mean oestrone and oestradiol concentrations before and during the last four hours of the infusion and the last two or four hours of the sampling period are shown in Table 2.12.

Prior to the infusion the plasma cortisol, androstenedione and testosterone concentrations were near the limits of sensitivity of the assays. During the oestradiol infusions, the plasma oestradiol levels rose, and eventually reached a plateau at about 15 ng/dl. Although the plasma oestrone concentrations rose in both subjects to 4-6 ng/dl shortly after the start of the infusions, the levels of both oestrone and oestradiol returned to their pre-infusion values.

The hormone profiles and mean plasma steroid concentrations in the subject (No. 34) who was infused oestrone are shown in Figure 2.40. and Table 2.12. respectively. Prior to the infusion, the plasma levels of both oestrogens were similar to those described above. During the oestrone infusion, the plasma oestrone levels rose to 24 ng/dl but there were marked fluctuations, and a plateau was not reached. Plasma oestradiol concentrations rose significantly ($P < 0.01$) from the pre-infusion mean value of 1.9 ± 0.6 ng/dl to a mean of 2.9 ± 0.7 ng/dl in the last four hours of the infusion. Both plasma oestrogens fell to their pre-infusion levels after the completion of the infusion.

Figure 2.38. Subject No. 32. Plasma oestrone (●), oestradiol (Δ), FSH (◆) and LH (◇) concentrations in blood samples obtained every 30 minutes from an ovariectomised woman after treatment with dexamethasone (Dex) 1.5 mg daily for one week, and when oestradiol was infused at 10 µg /hour for six hours.

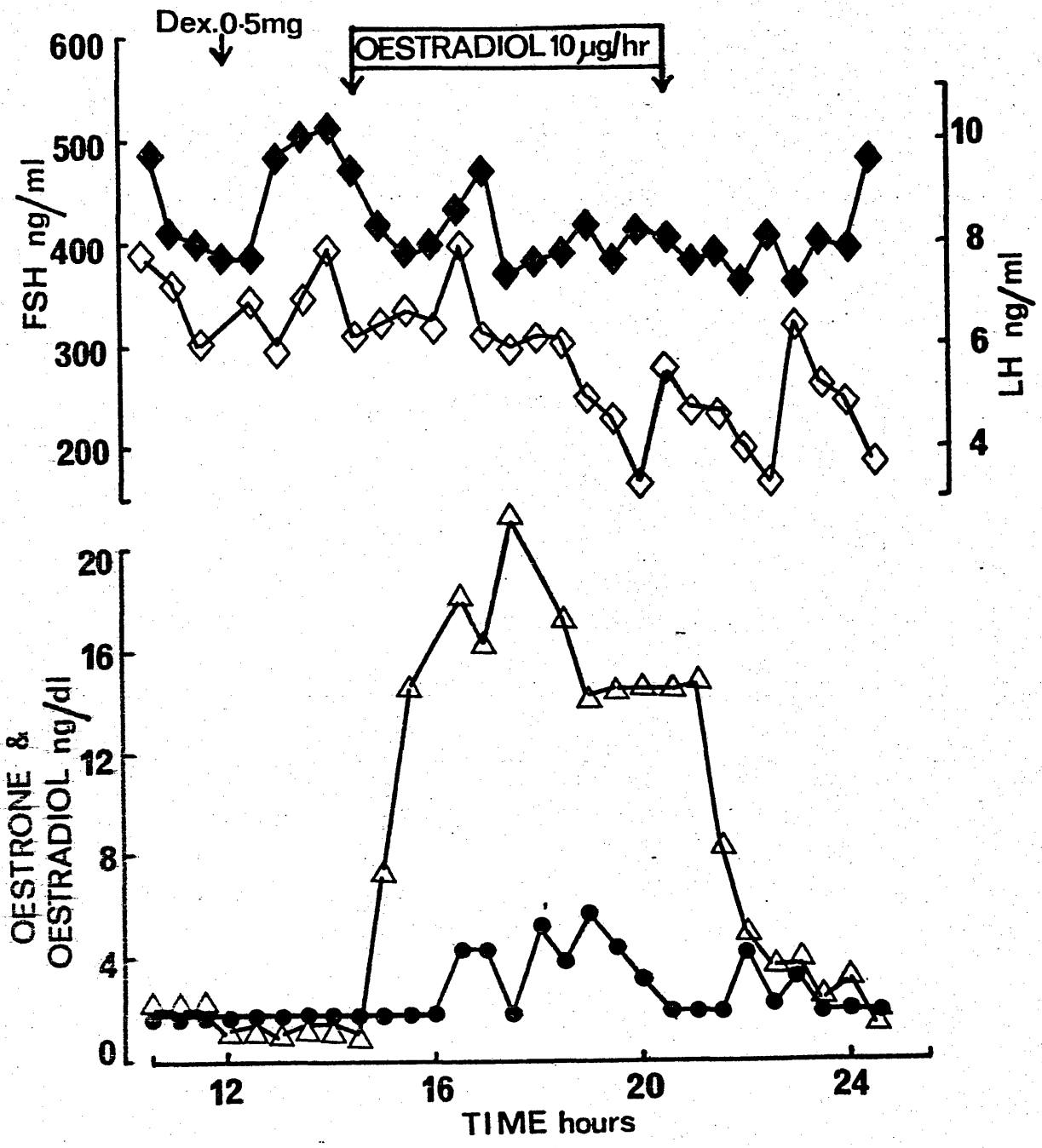

Dex.0·5mg
OESTRADIOL 10 µg/hr
FSH ng/ml
LH ng/ml
600
500
400
300
200
10
8
6
4
OESTRONE &
OESTRADIOL ng/dl
20
16
12
8
4
0
12
16
20
24
TIME hours

Figure 2.39. Subject No. 33. Plasma oestrone (●), oestradiol (△), FSH (◆) and LH (◇) concentrations in blood samples obtained every 30 minutes from a post-menopausal woman after treatment with dexamethasone 1.5 mg daily for one week, and when oestradiol was infused at 10 µg/hour for six hours.

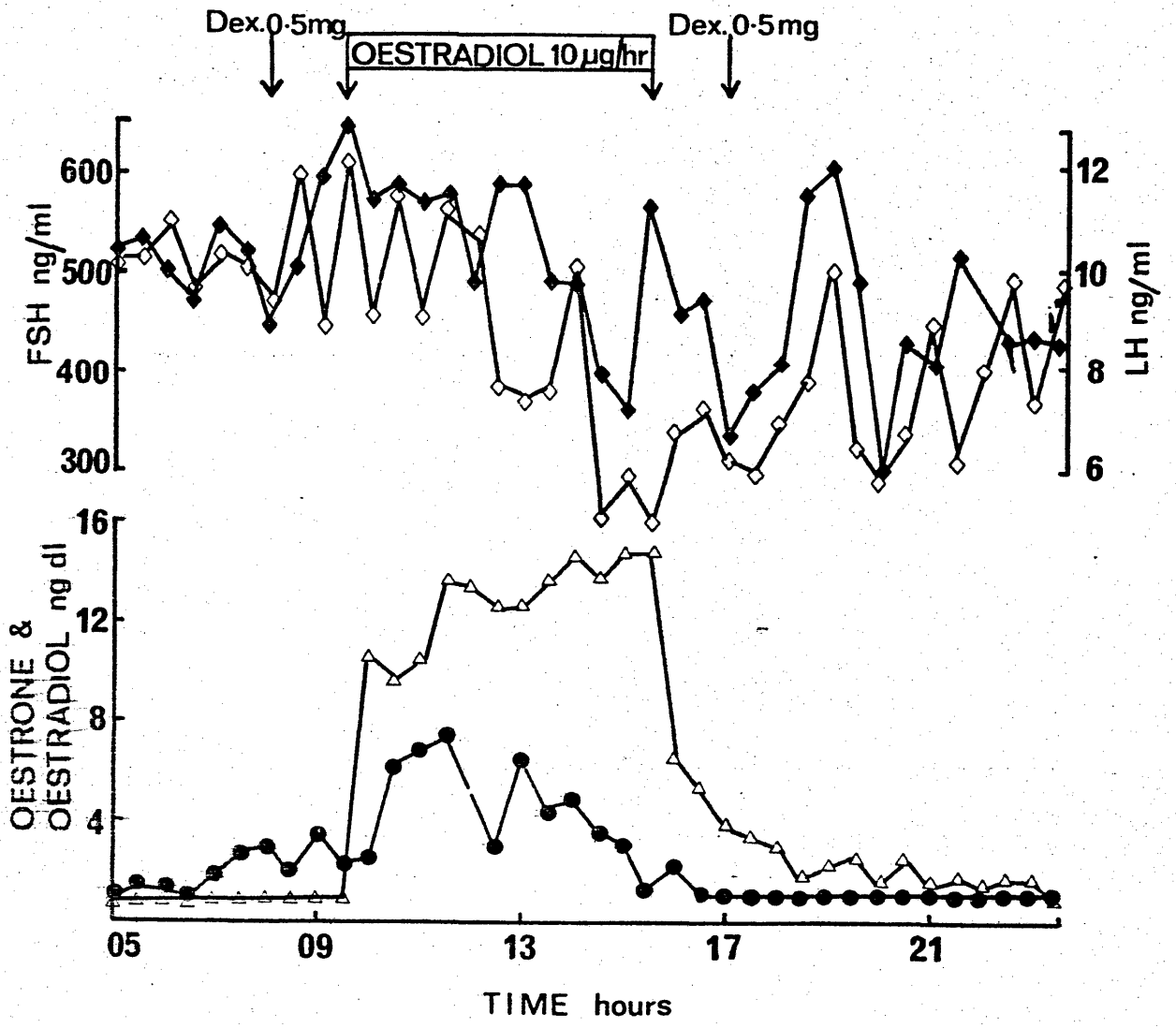
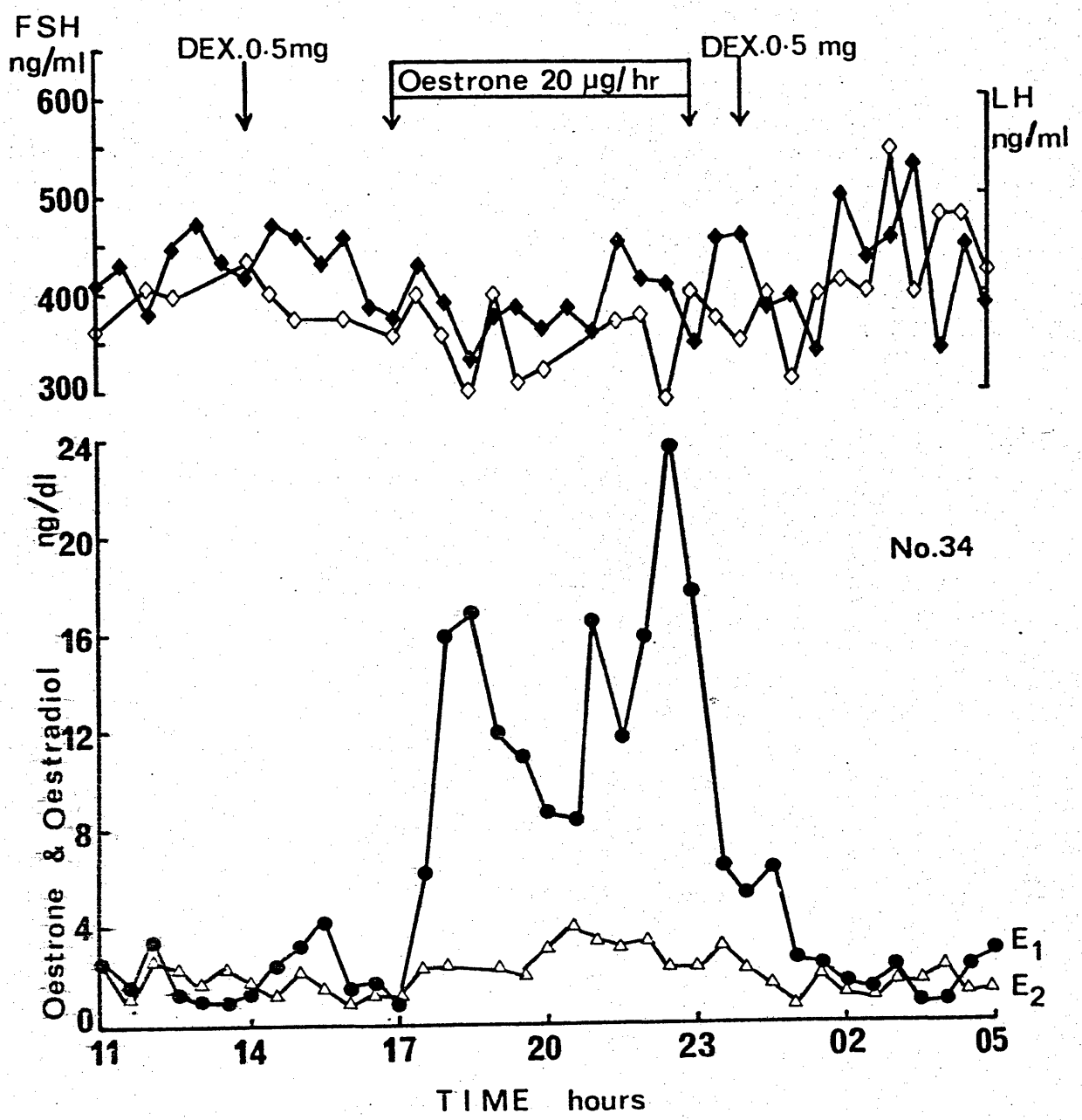

Dex.0·5mg
OESTRADIOL 10 µg/hr
Dex.0·5mg
FSH ng/ml
600
500
400
300
LH ng/ml
12
10
8
6
OESTRONE &
OESTRADIOL ng dl
16
12
8
4
05
09
13
17
21
TIME hours

Table 2.12. Mean oestrone (E_1), oestradiol (E_2), FSH and LH concentrations in blood samples obtained every 30 minutes before infusion (Pre), during the last four hours of a six hour infusion of oestradiol or oestrone, and in a two or four hour period after the infusion. All the women had had prior treatment with dexamethasone (1.5mg daily) for one week. The figures in brackets denote the hours after the completion of the infusion during which the sampling occurred. N. is the number of samples. (Significance of difference from pre-infusion levels: *** $p = < 0.001$, ** $p = < 0.01$, * $p = < 0.05$. (M) is Mann Whitney U test.

NO	SAMPLING VS INFUSION	N.	E_1 ng/dl	E_2 ng/dl	FSH ng/ml	LH ng/ml
	Pre	9	< 2.0	1.6 ± 0.4	447 ± 53	6.8 ± 0.7
32	During E_2	9	$3.8 \pm 1.3^{**(M)}$	$16.8 \pm 2.2^{***(M)}$	406 ± 42	$5.7 \pm 1.2^{*}$
	Post (2-4)	5	2.3 ± 0.6	$3.0 \pm 0.9^{*(M)}$	412 ± 46	$4.8 \pm 1.3^{**}$
	Pre	10	2.0 ± 0.9	< 1.0	533 ± 58	10.5 ± 1.0
33	During E_2	9	4.3 ± 1.9	$13.6 \pm 0.8^{***(M)}$	506 ± 83	7.9 ± 2.5
	Post (4-8)	9	$< 1.0^{*(M)}$	$1.7 \pm 0.5^{**(M)}$	$431 \pm 62^{**}$	$7.7 \pm 1.5^{***}$
	Pre	13	2.1 ± 1.0	1.9 ± 0.6	430 ± 34	7.8 ± 0.5
34	During E_1	9	$14.1 \pm 5.0^{***(M)}$	$2.9 \pm 0.7^{**}$	$391 \pm 36^{*}$	7.1 ± 0.9
	Post (2-6)	9	2.2 ± 0.7	1.8 ± 0.5	428 ± 67	$8.7 \pm 1.3^{*(M)}$

Figure 2.40. Plasma oestrone (E_1), oestradiol (E_2), FSH (♦) and LH (◇) concentrations in blood samples obtained every 30 minutes from an ovariectomised woman (Subject No. 34) after treatment with dexamethasone (Dex) 1.5 mg daily for one week, and when oestrone was infused at a rate of 20 µg/hour for six hours.


FSH
ng/ml
600
500
400
300
DEX.0·5mg
Oestrone 20 µg/hr
DEX.0·5 mg
LH
ng/ml
24
20
16
12
8
4
0
ng/dl
Oestrone & Oestradiol
No.34
E1
E2
11
14
17
20
23
02
05
TIME hours

iii) Effects of Body Weight

The plasma hormone profiles of the grossly obese postmenopausal woman (Subject No. 39) is shown in Figure 2.41. The mean plasma cortisol, androstenedione, testosterone, oestrone and oestradiol concentrations were 5.9 ± 4.4 µg/dl, 74.0 ± 32.0 ng/dl, 33.4 ± 8.1 ng/dl, 8.6 ± 1.3 ng/dl and 4.5 ± 0.9 ng/dl respectively. The mean oestrogen concentrations were at the upper limits (two standard deviations of the mean) of the range of concentrations found in postmenopausal women who were within 12% of the ideal weight for their height (Part 2 a). The other steroid concentrations and the patterns of all the steroid levels in this woman were similar to those usually seen in normal postmenopausal women.

The mean oestrone, oestradiol and testosterone concentrations and the body weights, percentage differences from ideal weight and the fat mass (the difference between ideal body weight for height and actual body weight) are shown in Table 2.4. The plasma oestradiol concentration correlated with body weight, and the percentage difference from the ideal weight as shown in Figures 2.42. and 2.43. respectively. The plasma oestrone concentration also correlated with the percentage difference from the ideal weight (Figure 2.44.). However the r values in these significant correlations of plasma oestrogens was about 0.4 and, furthermore, plasma oestrone levels did not correlate with actual body weight. Neither the mean plasma androstenedione concentration, nor the testosterone concentration correlated with body weight, or the percentage difference from ideal body weight.

iv) Effects of Thyroid Hormones

The plasma hormone profile of the hyperthyroid woman (Subject

Figure 2.41. Plasma hormone concentrations in blood samples obtained every 30 minutes for 24 hours from an obese postmenopausal woman (Subject No. 39) who weighed 107.5 Kg.

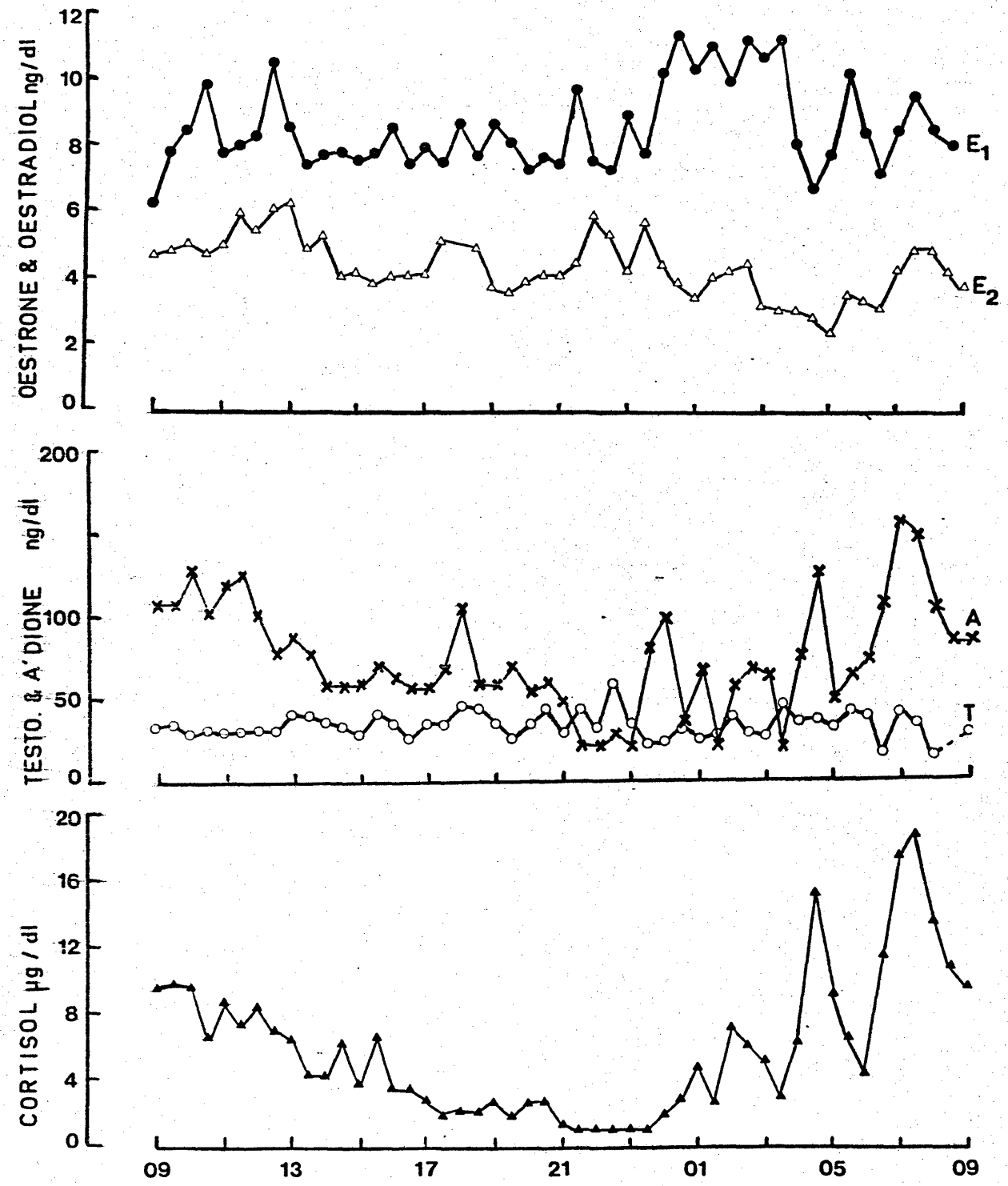

OESTRONE & OESTRADIOL ng/dl
12
10
8
6
4
2
0
E1
E2
TESTO. & A'DIONE ng/dl
200
100
50
0
A
T
CORTISOL µg / dl
20
16
12
8
4
0
09
13
17
21
01
05
09

Figure 2.42. Correlation of mean plasma oestradiol concentrations and body weight in 28 postmenopausal women or ovariectomised women.

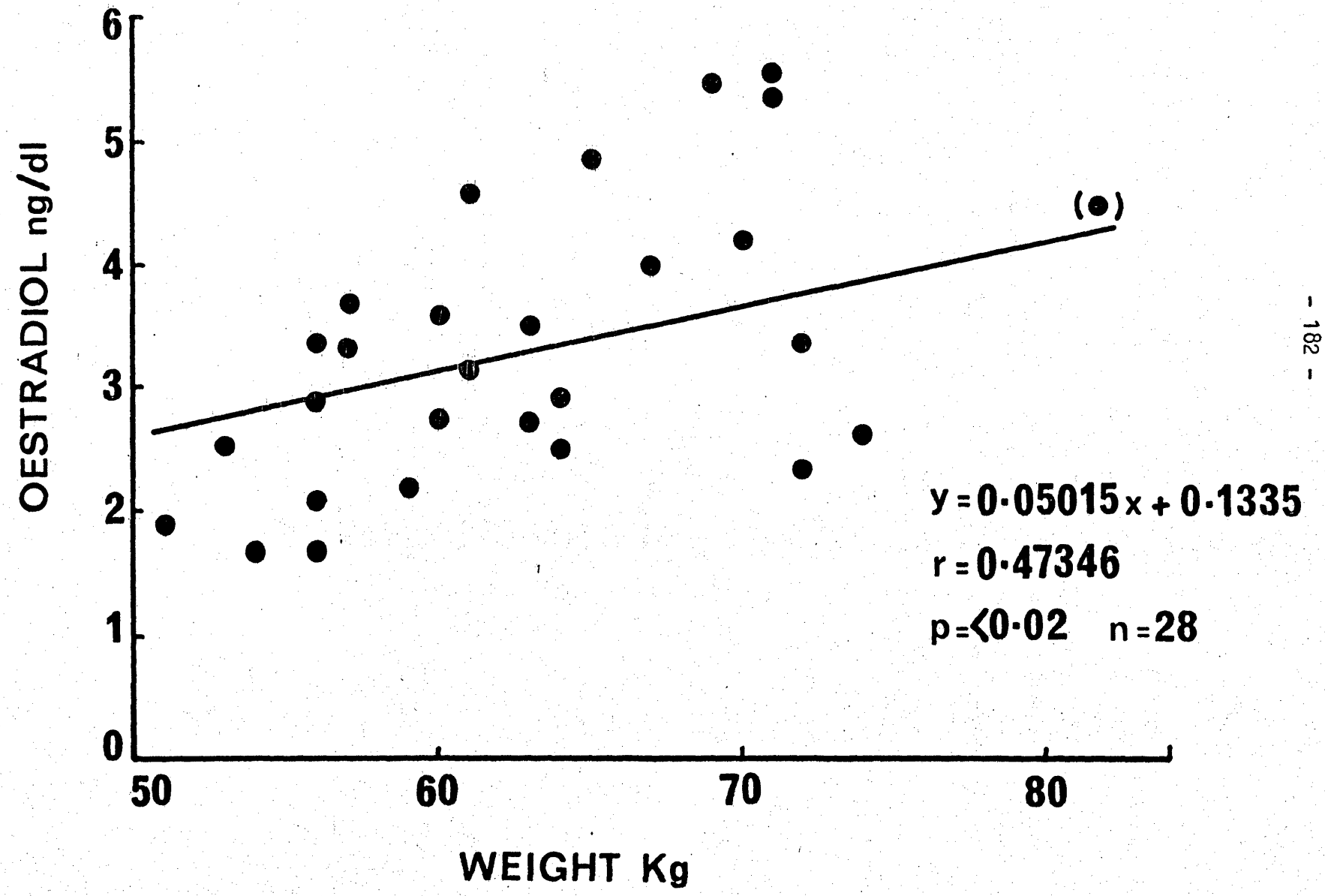

OESTRADIOL ng/dl
6
5
4
3
2
1
0
50
60
70
80
WEIGHT Kg
y = 0·05015 x + 0·1335
r = 0·47346
p = <0·02 n = 28

Figure 2.43. Correlation of mean plasma oestradiol concentration and percentage difference from the ideal body weight for their height of 28 postmenopausal women or ovariectomised women.

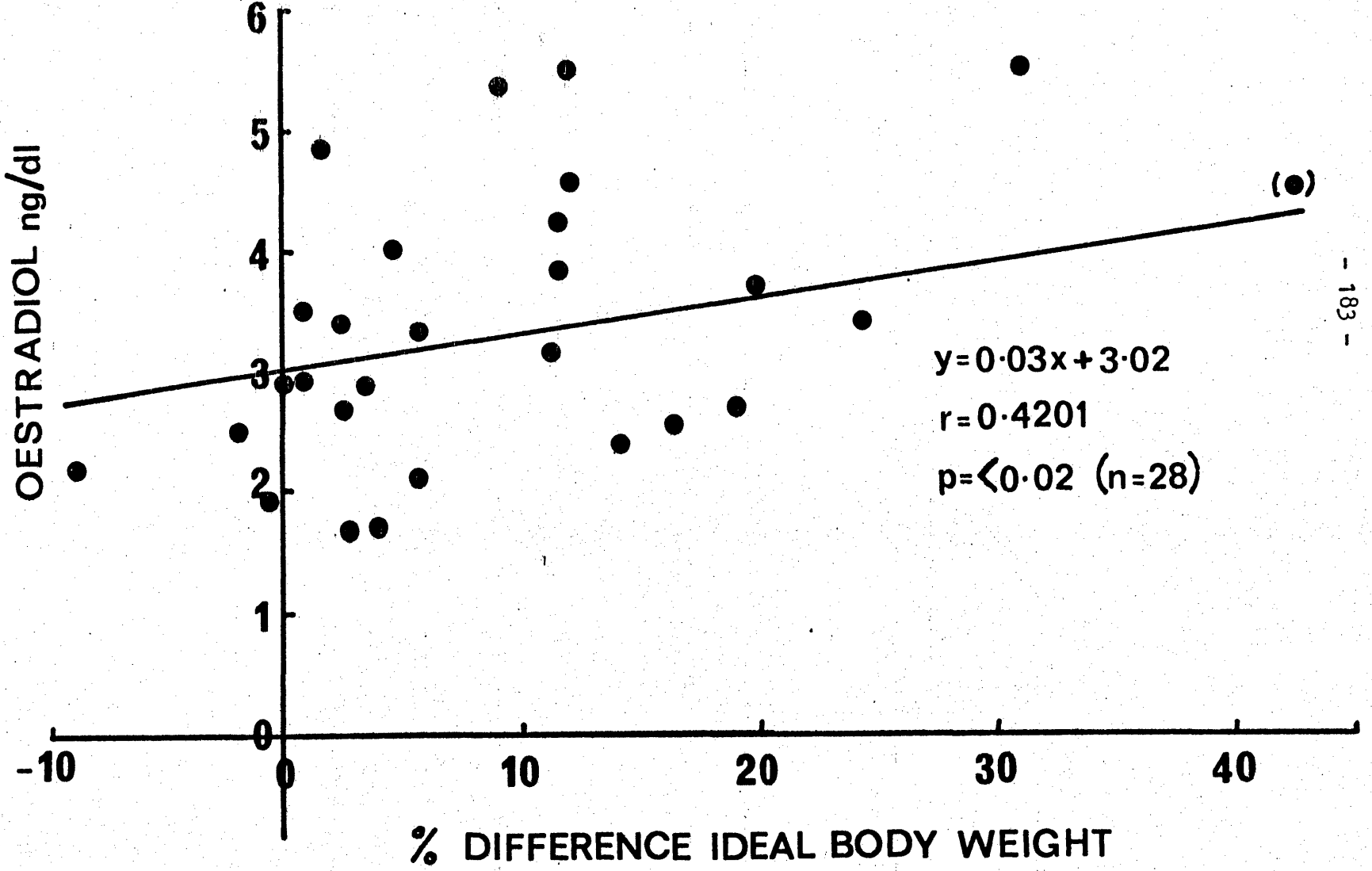
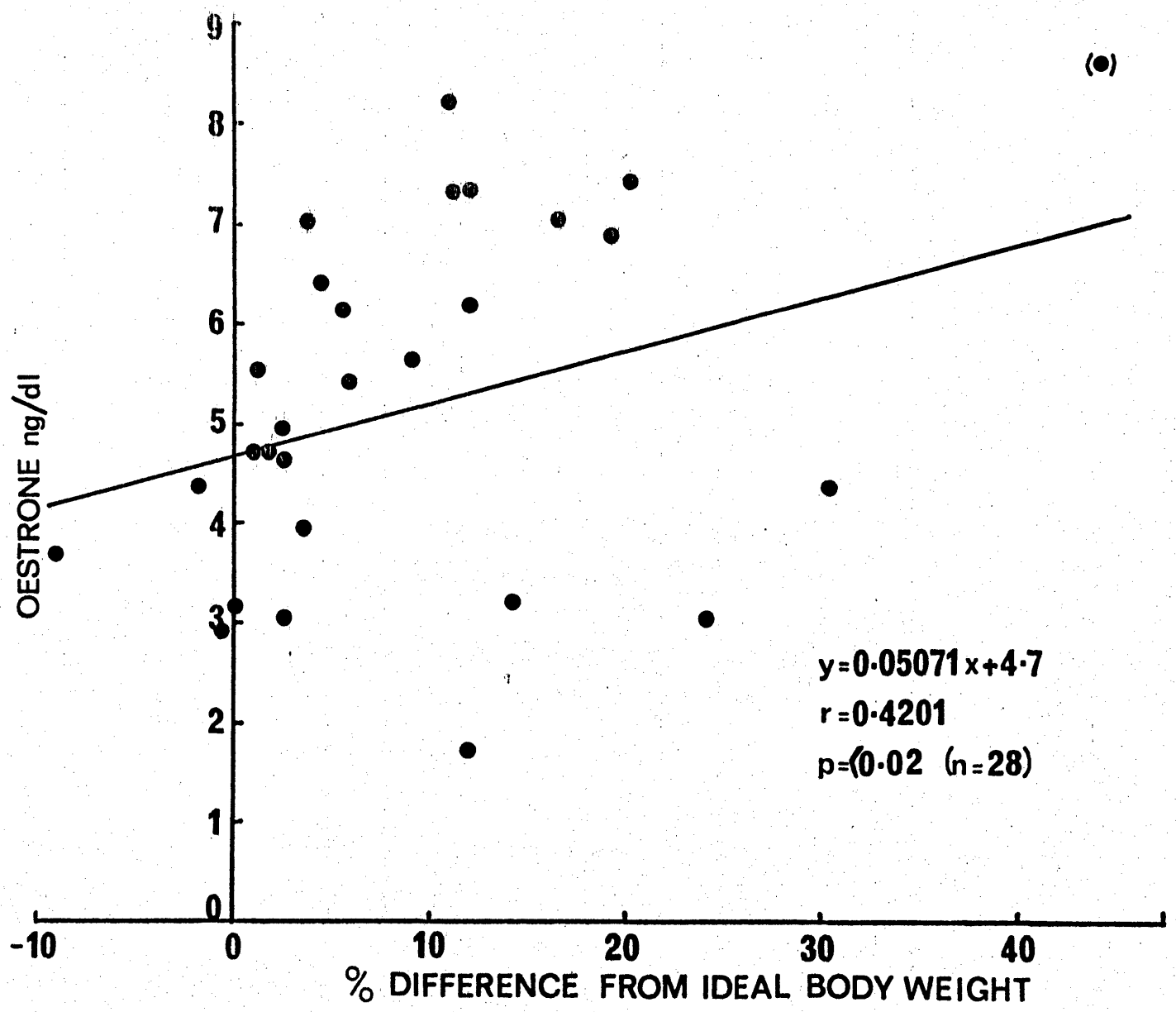

OESTRADIOL ng/dl
6
5
4
3
2
1
0
-10
0
10
20
30
40
% DIFFERENCE IDEAL BODY WEIGHT
y=0·03x+3·02
r=0·4201
p=<0·02 (n=28)

Figure 2.44. Correlation of mean plasma oestrone and percentage difference from the ideal body weight for their height for 28 postmenopausal women or ovariectomised women.


OESTRONE ng/dl
9
8
7
6
5
4
3
2
1
0
-10
0
10
20
30
40
% DIFFERENCE FROM IDEAL BODY WEIGHT
(●)
y=0·05071x+4·7
r=0·4201
p=(0·02 (n=28)

No. 47) is shown in Figure 2.45. The mean plasma oestradiol and oestrone concentration prior to the administration of the dexamethasone were 5.6 ± 1.9 ng/dl and 3.8 ± 2.1 ng/dl respectively. This oestradiol level, but not oestrone level, was abnormally high when compared with the levels in normal postmenopausal or ovariectomised women (Parts 2 a and 2 b). The oestradiol to oestrone ratio of 1.5:1.0 in this woman was the reverse of the ratio of 0.6:1.0 in normal postmenopausal women (Part 2 a). The mean plasma steroid concentrations between 0400 and 1000 hours (during the early morning surge in ACTH) are shown in Table 2.13.) both for the control night and for the following night after the dexamethasone treatment. The mean plasma cortisol, androstenedione, oestrone and oestradiol concentrations were significantly lower ($P < 0.001$) on the treatment night than on the control night, but mean testosterone concentrations were unchanged after the dexamethasone. The patterns of all the hormone concentrations were similar to those described in normal postmenopausal or ovariectomised women.

Six months after the study a random plasma oestradiol sample was collected and was 1.5 ng/dl.

v) Peripheral 'Stores'

The mean plasma testosterone, oestrone and oestradiol concentrations of each of the 26 postmenopausal or ovariectomised women are shown in Table 2.4. The mean testosterone, oestrone and oestradiol concentrations for this group of women were 35.6 ± 13.4 ng/dl, 5.1 ± 1.8 ng/dl and 3.2 ± 1.1 ng/dl respectively. The mean plasma cortisol and androstenedione concentrations in the eight normal postmenopausal or ovariectomised women in whom levels

Figure 2.45. Plasma cortisol (F), androstenedione (A), testosterone (T), oestrone (E_1) and oestradiol (E_2) concentrations in blood samples obtained every 30 minutes from a hyperthyroid woman (Subject No. 47) on a control night and the following night when dexamethasone (Dex) was administered at 2400 hours.

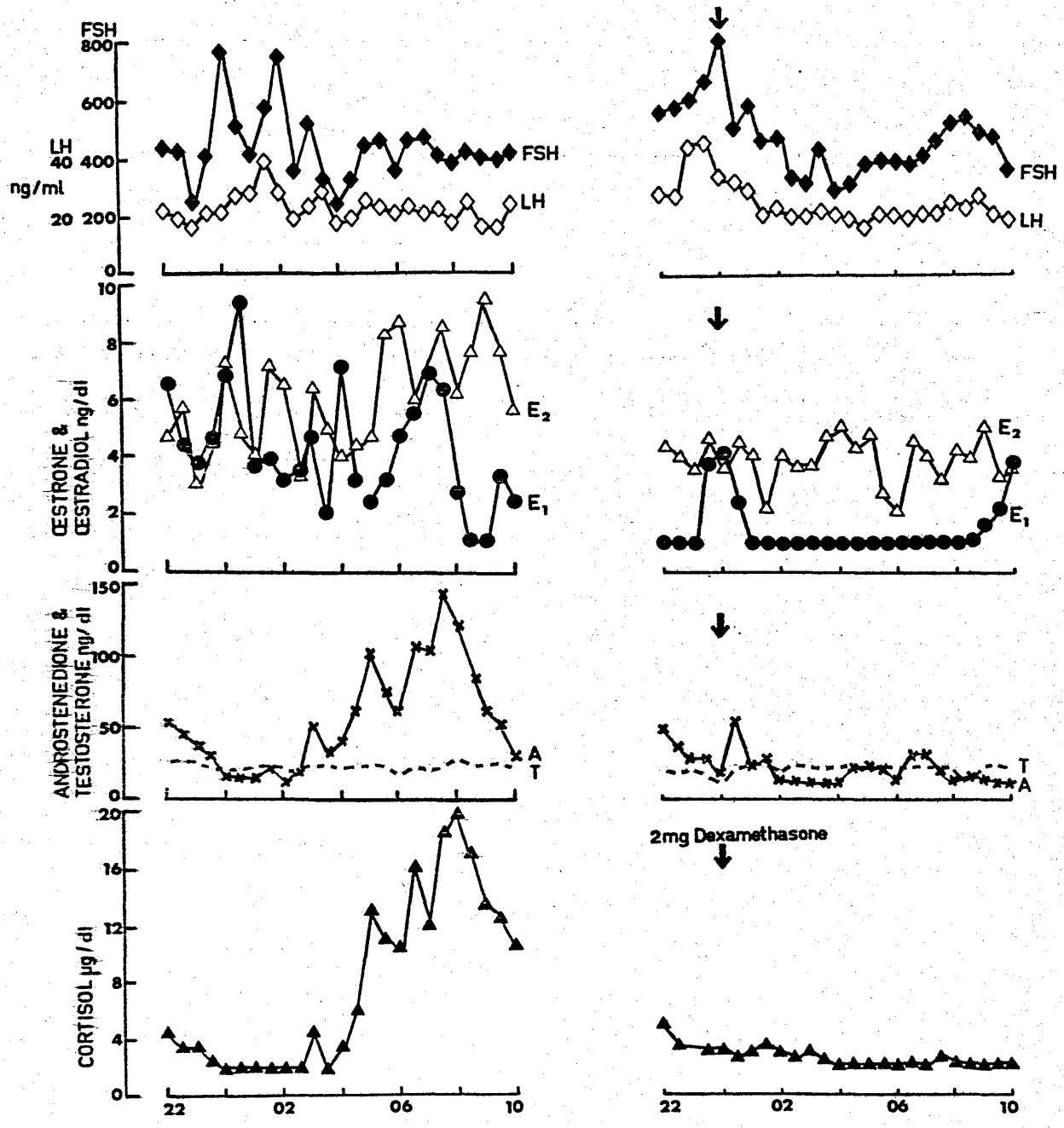

FSH
LH
ng/ml
FSH
LH
ŒSTRONE & ŒSTRADIOL ng/dl
E2
E1
ANDROSTENEDIONE & TESTOSTERONE ng/dl
A
T
CORTISOL µg/dl
2mg Dexamethasone
22
02
06
10

Table 2.13. Mean ± SD steroid and gonadotrophin concentrations in blood samples obtained from a hyperthyroid woman (Subject No. 47) every 30 minutes between 0400-1000 hours on a control morning (Pre-Dex), and the following morning (Post-Dex) after 2mg dexamethasone was administered at 2400 hours. (Significance of difference between 'Pre-Dex' and 'Post-Dex' levels: *** $p = <0.001$. (M) denotes the Mann Whitney U test.

SAMPLING PERIOD	F µg/dl	A ng/dl	T ng/dl	E_1 ng/dl	E_2 ng/dl	FSH ng/ml	LH ng/ml
Pre-Dex	12.6 ± 4.6	80.7 ± 33.7	22.6 ± 2.3	3.9 ± 2.1	6.8 ± 1.9	402 ± 64	21.6 ± 2.5
Post-Dex	1.6 ± 0.5***(M)	16.9 ± 8.3***(M)	21.2 ± 2.4	1.4 ± 0.9***	4.0 ± 0.9***(M)	416 ± 76	21.1 ± 2.5

were determined in blood samples obtained for 24 hours, are shown in Tables 2.7. and 2.8. respectively. Their mean cortisol and androstenedione concentrations were 13.4 ± 4.6 µg/dl and 104.1 ± 38.6 ng/dl respectively. The mean plasma steroid concentrations for the five women (Subject Nos. 6,20,26,16 & 25) after the single dose of dexamethasone are given in Table 2.6. and for the 11 women after treatment for one week with dexamethasone (Subject Nos. 7, 19, 21,27 - 34) in Table 2.5. The mean steroid concentrations for each group of women are detailed in Table 2.14. and shown in Figure 2.46. Plasma cortisol and androstenedione concentrations were significantly lower ($P < 0.001$) in both groups treated with dexamethasone, when compared with the levels in the control group. The mean plasma oestrone, but not testosterone or oestradiol, concentration was also significantly lower ($P < 0.05$) in the group of women studied after a single dose of dexamethasone. All steroid concentrations were significantly lower ($P < 0.001$) in the women treated with dexamethasone for one week when compared with the levels in the control group.

Four hours after the completion of the infusions of ACTH or androstenedione in Subject Nos. 7,19, 21, 27 and 28, the plasma androstenedione and cortisol concentrations had almost returned to their pre-infusion levels as shown in their respective figures (2.24.,2.25.,2.33., & 2.34.). The mean plasma testosterone, oestrone and oestradiol concentrations in the sampling periods before, during and after these infusions are given in Table 2.4. and shown in Figure 2.47. Plasma oestrone levels in samples obtained between the fourth and ninth hours after the completion of the infusions in Subject Nos. 27 and 28 were < 2 ng/dl. whereas the levels in

Table 2.14. Mean ± SD steroid concentrations in three groups of women: those not prescribed dexamethasone, those studied after a single dose of dexamethasone was administered at 2400 hours and those studied after dexamethasone treatment (1.5 mg daily) for one week. n = no. of women studied. The mean concentrations determined are in blood samples obtained every 30 minutes for 4-24 hours for testosterone, oestrone and oestradiol concentrations in the control group, otherwise for four hours between 0430 & 0830. (Significance of differences from levels in the control group: *** p = <0.001, ** p = < 0.01, * p = <0.05. (M) denotes the Mann Whitney U test.)

GROUP	F µg/dl	A ng/dl	T ng/dl	E_1 ng/dl	E_2 ng/dl
No dexamethasone	13.4 ± 4.6 (0430-0830, n = 8)	104.1 ± 38.6	35.6 ± 13.4 (n = 26)	5.1 ± 1.8 (n = 26)	3.2 ± 1.1 (n = 26)
Dexamethasone 1 dose (n = 5)	1.8 ± 0.8 ***(M)	31.8 ± 5.3 ***(M)	25.1 ± 13.0	2.7 ± 1.4	3.2 ± 1.6
Dexamethasone 1 week (n = 11)	1.0 ± 0.1 ***(M)	26.9 ± 0.4 ***(M)	14.6 ± 7.9 ***(M)	1.6 ± 0.6 ***(M)	1.8 ± 0.5 ***(M)

Figure 2.46. Mean plasma cortisol (F), androstenedione (A), testosterone (T), oestrone (E_1) and oestradiol (E_2) concentrations in postmenopausal or ovariectomised women not prescribed dexamethasone (No Dex) or after treatment with dexamethasone (Dex) for one day (1) when a 2 mg dose was given, or for seven days (7) during which 1.5 mg daily was prescribed. (*** $P < 0.001$; ** $P < 0.01$).

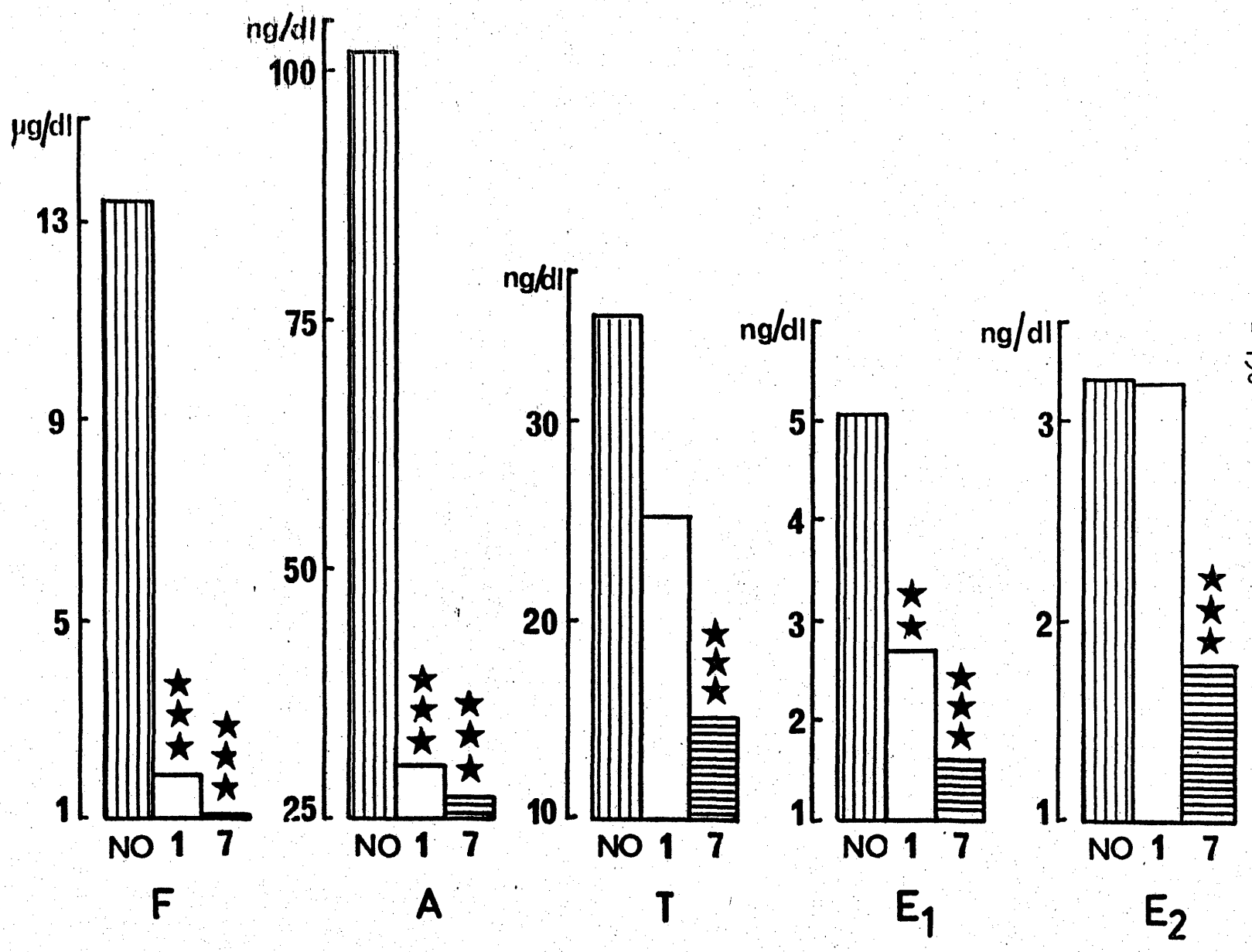
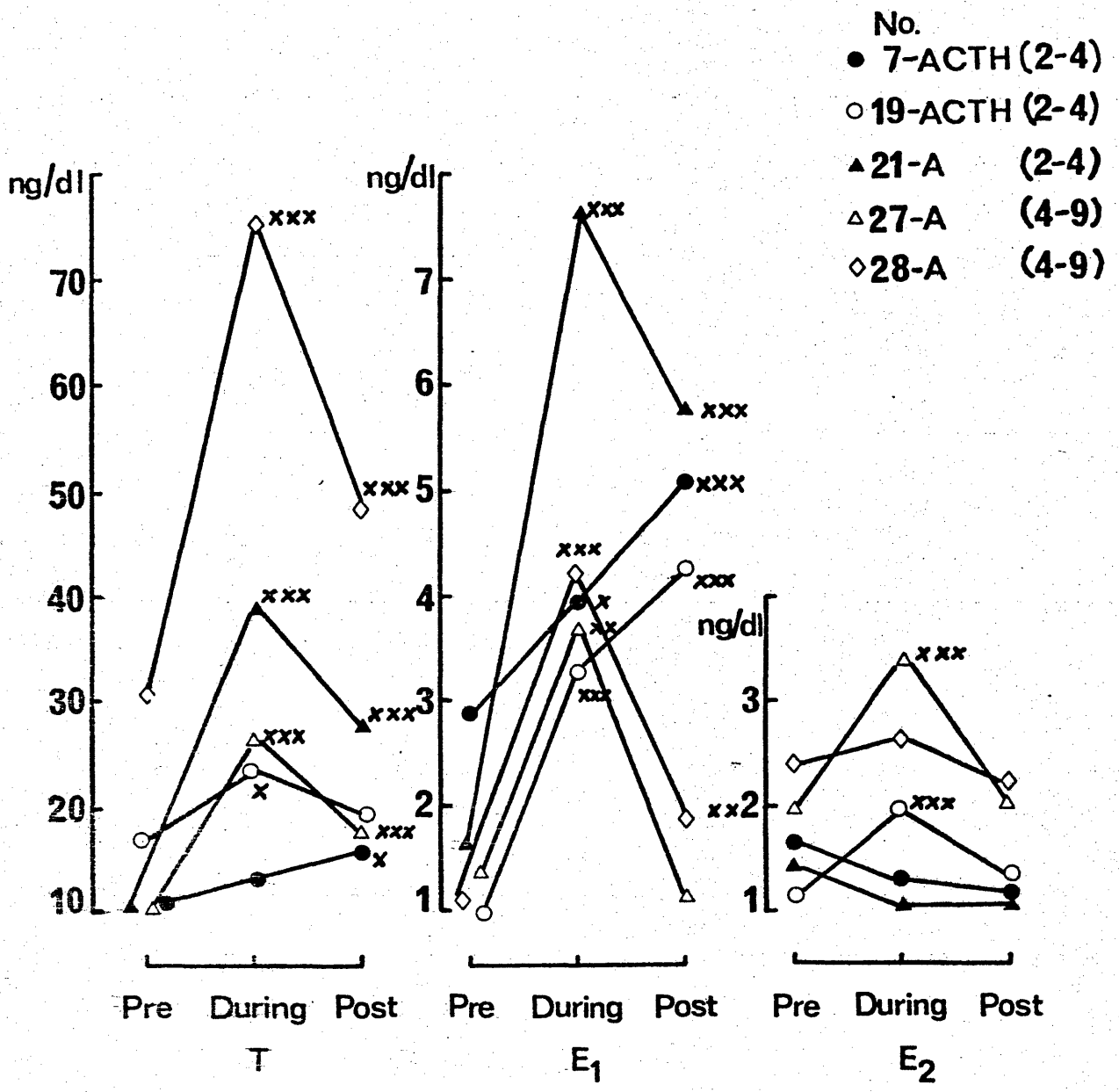

µg/dl
13
9
5
1
NO 1 7
F
ng/dl
100
75
50
25
NO 1 7
A
ng/dl
30
20
10
NO 1 7
T
ng/dl
5
4
3
2
1
NO 1 7
E1
ng/dl
3
2
1
NO 1 7
E2

Figure 2.47. Mean testosterone (T), oestrone (E_1) and oestradiol (E_2) concentrations in five subjects (studied after one week of treatment with dexamethasone) before (pre) and during the last four hours of a ~~six hour~~ infusion of tetracosactrin (ACTH) or androstenedione (A), and in a two or five hour sampling period (Post) beginning two or four hours after the completion of the infusion. The figures in brackets denote the start and finish hours of the post-infusion sampling period. (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)


No.
● 7-ACTH (2-4)
○ 19-ACTH (2-4)
▲ 21-A (2-4)
△ 27-A (4-9)
◇ 28-A (4-9)
ng/dl
70
60
50
40
30
20
10
ng/dl
7
6
5
4
3
2
1
ng/dl
3
2
1
Pre During Post
T
Pre During Post
E1
Pre During Post
E2

the other three women were only obtained in the second to fourth hours and were >4 ng/dl. The mean post-infusion level of plasma testosterone was significantly higher ($P<0.001$) than the pre-infusion level in all three subjects infused androstenedione. The mean plasma oestradiol concentrations before and after the infusions were similar.

In two of the three subjects infused testosterone, the pre- and post-infusion concentrations of testosterone were not significantly different from each other (Figure 2.48.) but in the third subject (No. 30), the mean testosterone levels in the second to fourth hours after the infusion were significantly higher ($P<0.001$) than pre-infusion levels. The effect of the testosterone infusions on mean post-infusion levels of oestrone and oestradiol are shown in Figure 2.49. After the infusions the mean plasma oestrone concentrations were all >2 ng/dl whereas the plasma oestradiol levels after the infusions were all similar to the pre-infusion levels. The mean plasma concentrations of oestrone and oestradiol after the infusion of either oestrogen, were near the limits of detection of the assays in two of the three subjects (Subject Nos. 32-34 - Table 2.15.) In one subject (No. 32) however, the oestradiol value in the second to fourth hours after the infusion was significantly higher ($P<0.05$) than the pre-infusion level.

DISCUSSION

a) Interconversion of Androgens: Horton and Tait (1966) showed by isotopic techniques that in premenopausal women about 14% of plasma androstenedione was converted to testosterone whereas the amount of plasma testosterone converted to androstenedione was

Figure 2.48. Mean testosterone (T) concentrations in three subjects before (Pre) and during the last four hours of a testosterone infusion, and in a four hours sampling period (Post) beginning two or three hours after the completion of the infusion. The mean oestrone (E_1) or oestradiol (E_2) concentrations in three other subjects before (Pre) and during the last four hours of infusions of either oestrone or oestradiol respectively and in a two or four hour sampling period (Post) beginning two hours after the completion of the infusion. ([ ] denotes the sampling period in hours after the completion of the infusion; *** $P < 0.001$, ** $P < 0.01$ * $P < 0.05$)

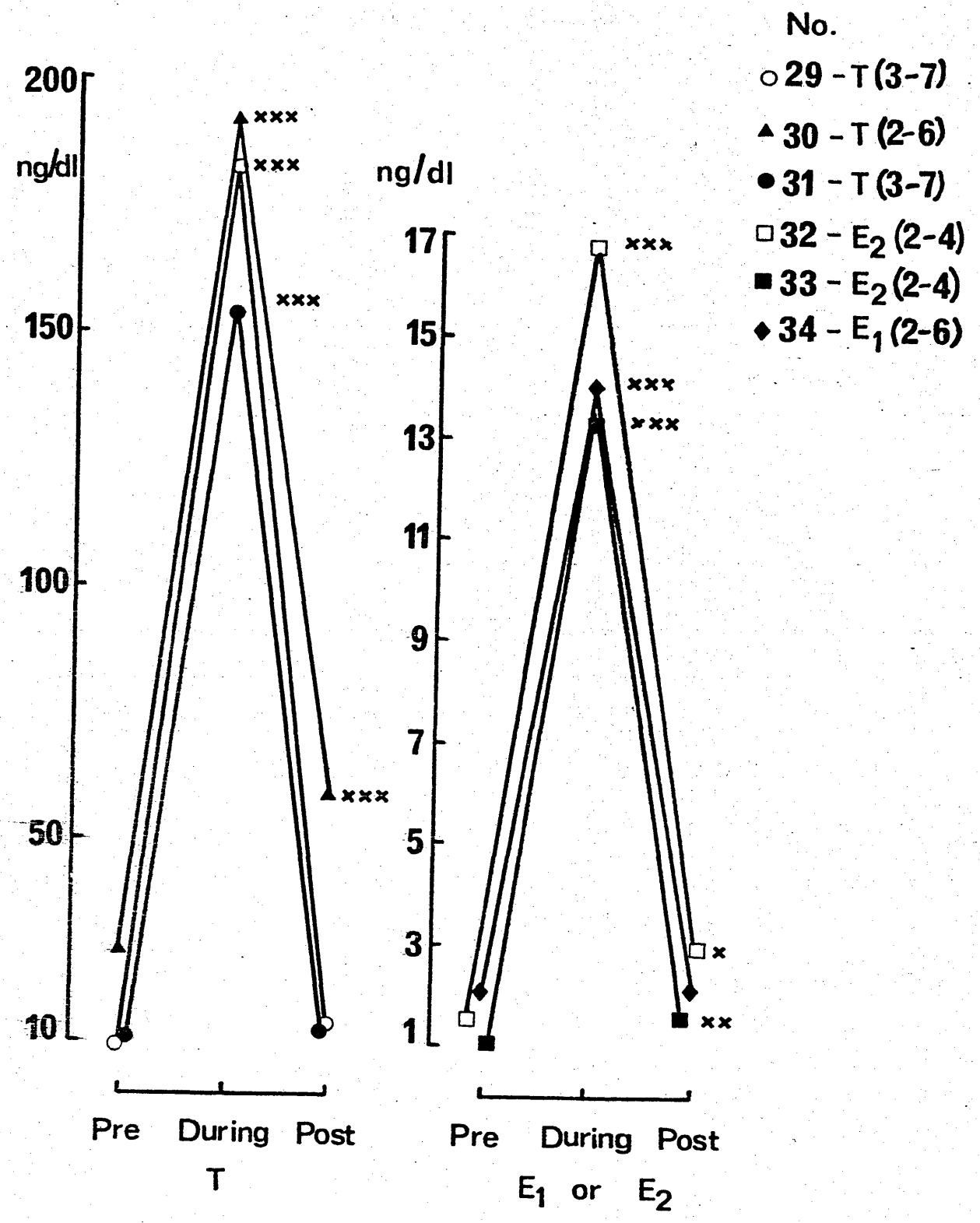

No.
○ 29 - T (3-7)
▲ 30 - T (2-6)
● 31 - T (3-7)
□ 32 - E_2 (2-4)
■ 33 - E_2 (2-4)
◆ 34 - E_1 (2-6)
200
ng/dl
150
100
50
10
×××
×××
×××
×××
ng/dl
17
15
13
11
9
7
5
3
1
×××
×××
×××
×
××
Pre
During
Post
T
Pre
During
Post
E_1 or E_2

Figure 2.49. Mean (product) oestrone (E_1) or oestradiol (E_2) concentrations in subjects before (Pre) and during the last four hours of a six hour in fusion of (precursor) testosterone (T), oestrone or oestradiol, and also in a two or four hour sampling period (start-finish hours) after the completion of the infusion (Post). *** $P < 0.001$; ** $P < 0.01$.

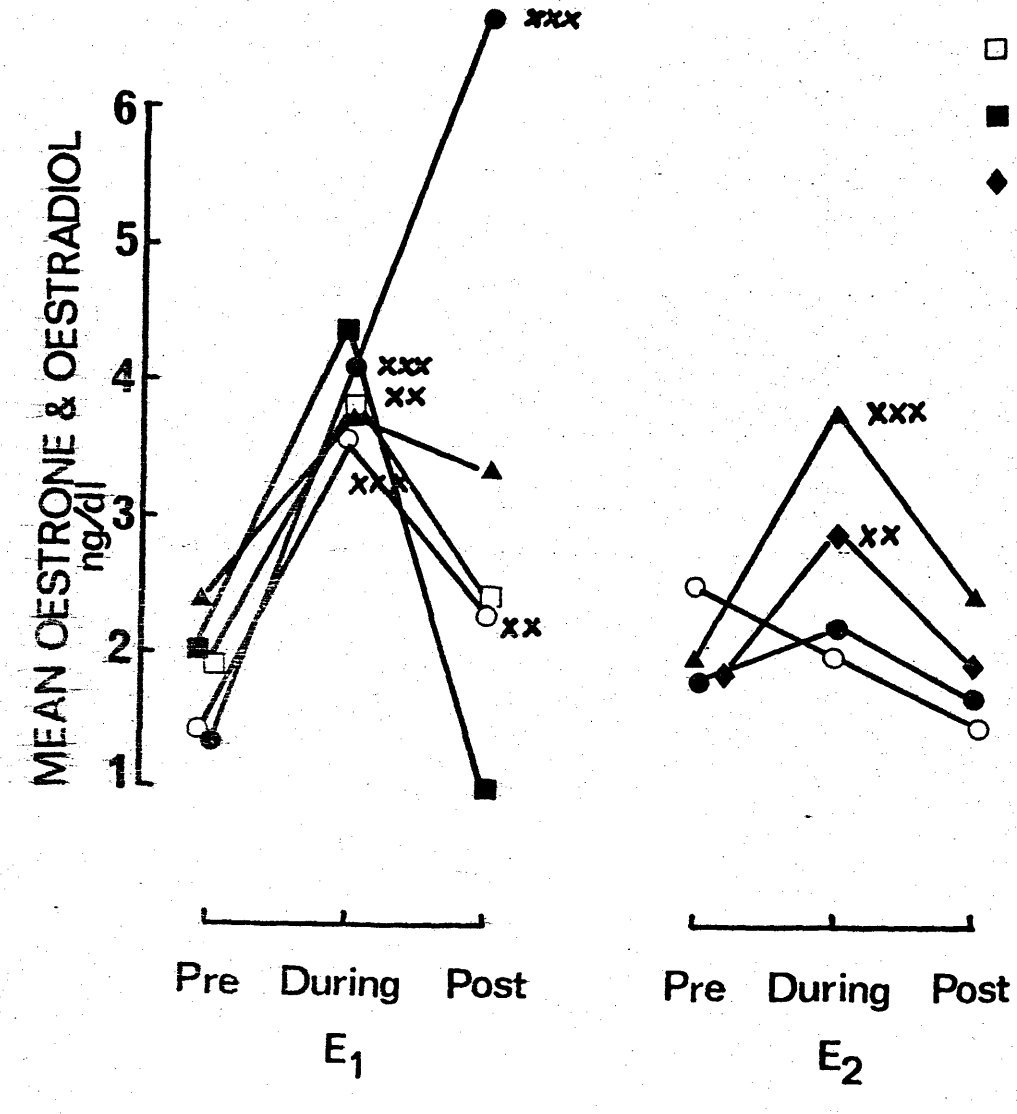

No.
○ 29 - T (3-7)
▲ 30 - T (2-6)
● 31 - T (3-7)
□ 32 - E2 (2-4)
■ 33 - E2 (2-4)
◆ 34 - E1 (2-6)
MEAN OESTRONE & OESTRADIOL ng/dl
6
5
4
3
2
1
xxx
xx
xxx
xx
xxx
xx
Pre During Post
E1
Pre During Post
E2

Table 2.15. Mean ± SD plasma testosterone (T), oestrone (E_1) and oestradiol (E_2) concentrations (ng/dl) in blood samples obtained every 30 minutes before infusion (Pre), during the last four hours of an infusion of tetracosactrin (ACTH), androstenedione (A), androstenedione plus cortisol (A + F), testosterone, oestrone or oestradiol, and after the infusion (Post). All subjects had had dexamethasone daily for one week prior to the study (1.5 mg daily). The figures in brackets denote the hours after the completion of the infusion during which the sampling occurred. (Significance of difference from pre-infusion levels: *** p = < 0.001, ** p = < 0.01, * p = < 0.05. (M) denotes the Mann Whitney U test). N. = no. of samples.

NO	SAMPLING PERIOD	N.	T	E_1	E_2
	Pre	9	11.0 ± 1.4	2.9 ± 0.5	1.7 ± 0.7
7	During ACTH	9	13.1 ± 3.1	4.0 ± 1.1*	1.3 ± 0.2
	Post (2-4)	5	16.0 ± 4.0*(M)	5.1 ± 1.0***	1.3 ± 0.5
	Pre	9	16.8 ± 6.3	1.0 ± 0.1	1.2 ± 0.3
19	During ACTH	9	24.1 ± 7.5*	3.3 ± 0.7*	2.0 ± 0.4***
	Post (2-4)	5	19.0 ± 6.4	4.3 ± 0.4	1.3 ± 0.2
	Pre	18	<10.0	1.6 ± 0.9	1.4 ± 0.4
21	During A	9	39.0 ± 6.9***(M)	8.1 ± 1.5***	<1.0
	Post (2-4)	4	27.5 ± 2.1***(M)	5.8 ± 0.9***	<1.0
	Pre	18	10.5 ± 0.7	1.3 ± 0.4	2.1 ± 0.5
27	During A + F	9	26.4 ± 5.6***(M)	3.8 ± 1.3***(M)	3.4 ± 0.6***
	Post (4-9)	11	17.5 ± 2.3***(M)	1.1 ± 0.4	2.1 ± 0.6
	Pre	19	31.4 ± 8.9	1.2 ± 0.3	2.6 ± 0.6
28	During A	9	77.2 ± 22.2***(M)	4.3 ± 1.2***(M)	2.7 ± 0.2
	Post (4-9)	11	49.2 ± 7.0***	1.8 ± 0.7**(M)	2.2 ± 0.5
	Pre	19	<10.0	1.4 ± 0.8	2.0 ± 0.6
29	During T	9	183.9 ± 29.3***(M)	3.5 ± 1.1***	2.0 ± 0.5
	Post (3-7)	9	15.2 ± 7.3	2.3 ± 0.8**	1.5 ± 0.4

cont.

table 2.15. (cont.)

NO	SAMPLING PERIOD	N.	T	E_1	E_2
	Pre	9	28.0	2.4 ± 0.6	2.5 ± 0.4
30	During T	9	193.0 ± 13.0***(M)	3.6 ± 1.4	3.8 ± 0.5***
	Post (2-6)	9	60.0 ± 12.0***(M)	3.4 ± 1.2	2.4 ± 0.5
	Pre	14	<10.0	1.3 ± 0.3	1.9 ± 0.4
31	During T	9	156.0 ± 21.3***(M)	4.1 ± 1.4***(M)	2.2 ± 0.7
	Post (3-7)	9	12.7 ± 3.6	6.7 ± 0.7***(M)	1.6 ± 0.7
	Pre	9		<2.0	1.6 ± 0.4
32	During E_2	9		3.8 ± 1.3**(M)	16.8 ± 2.2***(M)
	Post (2-4)	5		2.3 ± 0.6	3.0 ± 0.9*(M)
	Pre	10		2.0 ± 0.9	1.0
33	During E_2	9		4.3 ± 1.9	13.6 ± 0.8***(M)
	Post (2-4)	9		<1.0	1.7 ± 0.5**(M)
	Pre	13		2.1 ± 1.0	1.9 ± 0.6
34	During E_1	9		14.1 ± 3.0***(M)	2.9 ± 0.7**
	Post (2-6)	9		2.2 ± 0.7	1.8 ± 0.5

insignificant in relation to the total production of androstenedione. In the present study, the ratio of the increases in the plasma androstenedione concentrations to the increases in the plasma testosterone concentrations during infusions of androstenedione (Figures 2.25., 2.33. & 2.34) or testosterone (Figures 2.35, 2.36 & 2.37) in the women studied after prior treatment with dexamethasone, was consistent with the above findings of Horton and Tait. Thus, conversion of androstenedione to testosterone as found by Horton and Tait (1966) in premenopausal women, also occurs in post-menopausal women, as found by Calanog et al. (1977). The results of the present study also showed that peripheral oxidation of testosterone is quantitatively insignificant when compared with the adrenal production of androstenedione. As previous studies have suggested that there is no adrenal secretion of testosterone (Parts 2 a & c), the positive correlation of the mean plasma androstenedione and testosterone concentrations in the present study suggests that a large amount of circulating testosterone in postmenopausal women may be derived from the peripheral conversion of androstenedione. However, as there was no diurnal variation in plasma testosterone levels (Part 2 a), further studies are required to determine the mechanisms regulating the production of plasma testosterone from androstenedione after the menopause, especially as there may be significant contributions by other possible precursors such as dehydroepiandrosterone, dehydroepiandrosterone sulphate and androstenediol (Mahesh and Greenblatt, 1962; Horton and Tait, 1966 & 1967).

It is not known for certain which peripheral tissues predominantly convert plasma androstenedione to testosterone,

although this probably occurs in extra-splanchnic tissues (Horton and Tait, 1966; Rivarola et al., 1967). As the plasma androgen concentrations in this study did not correlate with fat mass, and the levels in the obese women were similar to those occurring in postmenopausal women of about ideal weight, the amount of adipose tissue is unlikely to affect the peripheral production of plasma testosterone after the menopause. A similar conclusion was drawn by Calanog et al. (1977) from the results of isotopic studies on the production rate of androstenedione and the conversion ratio of androstenedione to testosterone in postmenopausal women.

Plasma testosterone concentrations in the study of the hyperthyroid woman were similar to those seen in the normal postmenopausal woman. This finding is, however, not in agreement with the results of isotopic studies of hyperthyroid premenopausal women by Southren et al. (1974) that showed that plasma levels of testosterone and the conversion ratio of androstenedione to testosterone were greater than in euthyroid premenopausal women. As the production and metabolism of testosterone in pre- and postmenopausal women may be different (Southren et al., 1968; Zumoff et al., 1976), the effects of thyroid hormone on the production of plasma androgens after the menopause requires further study.

b) Aromatisation of Plasma Androgens: After West et al. (1956) first demonstrated extraglandular conversion of androgens to oestrogens, MacDonald et al. (1967) showed that about 40 ug/day of oestrone was normally produced in women from plasma androstenedione in extraglandular sites. Longcope (1971) and

Grodin et al. (1973) subsequently showed in their isotopic studies that nearly all plasma oestrone in postmenopausal women could be accounted for by the peripheral conversion of plasma androstenedione, a finding which was also consistent with the results of the androstenedione infusions in the present study. The infusions of unlabelled androstenedione or testosterone also showed that the degree of aromatisation of androstenedione was much greater than that of testosterone in postmenopausal or ovariectomised women. This conclusion is consistent. with the findings of isotopic studies of premenopausal women by Longcope et al. (1968) and Olivo et al. (1973), both of whom also observed that androstenedione and testosterone were converted preferentially to oestrone and oestradiol respectively. The results of the present study confirm peripheral aromatisation of androstenedione to oestrone, but conversion to oestradiol during the infusions of testosterone occurred only in one postmenopausal woman (Subject No. 30 - Figure 2.36.). In the other two women, who were ovariectomised, there was a preferential increase in the plasma oestrone concentrations during the infusion. Longcope et al. (1968) could not detect any aromatisation of testosterone in six premenopausal women and Olivo et al. (1973) found that the mean conversion ratio of testosterone to oestrone in six premenopausal women was half that of testosterone to oestradiol. The cause of the discrepancy in the results of these studies in premenopausal women, and of the present infusion studies in postmenopausal women treated with dexamethasone is presently unclear. Further studies of the factors that control the rate of aromatisation are obviously required. In the present study, the conversion of testosterone to oestrone would require that the

postulated intermediary (androstenedione) did not appear in the circulation.

Isotopic studies by Siiteri and MacDonald (1973), Rizkallah et al. (1975) and MacDonald et al. (1978) have all shown a correlation of the amount of conversion of androstenedione to oestrone and the body weight of postmenopausal women. In vitro studies (Schindler et al., 1972; Nimrod and Ryan, 1975) have demonstrated that adipose tissue has the enzymatic ability to convert androstenedione to oestrone. Thus, it has been concluded that adipose tissue is the major site of production of oestrogens in postmenopausal women. This conclusion is supported by the findings in this study of positive correlations of the percentage difference from ideal body weight (reflecting fat mass) and the plasma oestrone or oestradiol concentrations in the postmenopausal or ovariectomised women, and of the increased plasma oestrogens in the obese woman. The positive correlations of plasma oestradiol and oestrone concentrations and body weight in this study, was also evident in the study by Judd et al. (1976), provided women prescribed oestrogen therapy were excluded from the analysis. However, the results of the studies by Pelc et al. (1978) contrast with the conclusions of the present study, and of the other studies described above, by suggesting that the production of oestrone after the menopause depends not on body weight but only on the amount of plasma androstenedione produced. The positive correlation between plasma androstenedione and oestrone in this study is perhaps suprising because the androstenedione concentration in a blood sample obtained at 0900 hours does not accurately reflect mean androstenedione levels over 24 hours in postmenopausal or

ovariectomised women (Appendix V) because of the significant diurnal variation of the level of this steroid.

The findings in the woman with hyperthyroidism of raised plasma oestradiol, but not oestrone, concentrations is not consistent with the results of isotopic studies in which androstenedione or testosterone was infused into hyperthyroid and euthyroid premenopausal women by Southren et al. (1974). These workers found that hyperthyroidism was associated with increased peripheral production of oestrone rather than oestradiol. However, the preferential increase in the oestradiol levels in the hyperthyroid woman in the present study may have occurred because hyperthyroidism is associated with a marked increase in the plasma levels of sex-hormone-binding globulin (which binds oestradiol rather than oestrone) and with a decreased metabolic clearance rate of oestradiol (Ridgway et al., 1975).

Until Smuk and Schwers (1977) demonstrated in vitro aromatisation in the human adult liver, it was widely assumed that hepatic aromatisation of androgens did not occur after birth. Recently drugs such as aminoglutethimide have also been shown to affect aromatisation (Santen et al., 1978). Increased peripheral aromatisation of androstenedione to oestrone in women with endometrial carcinoma, when compared with other postmenopausal women has been reported by Siiteri and MacDonald (1973), Häusknecht and Gusberg (1973) and Calanog et al. (1977), although recent extensive studies by MacDonald et al. (1978) have not confirmed this finding. Possible roles for oestrogens after the menopause in the genesis of endometrial carcinoma that have not yet been researched, are the conversion of androsgens to plasma oestrogens other than oestrone, or the

aromatisation of plasma oestrogens in the endometrium. Clearly, further studies of the factors influencing the rate of aromatisation are necessary to increase our present understanding of the extraglandular production of plasma oestrogens after the menopause.

c) Interconversion of Plasma Oestrogens: The findings in the present study of a larger increase in plasma oestrone levels during the oestradiol infusions than the increase in plasma oestradiol during the oestrone infusion, are consistent with the conversion ratios reported by Longcope et al. (1968), albeit in premenopausal women. This interconversion may occur in several peripheral tissues (Ryan and Engel, 1952). The factors regulating such production are presently unknown but they may be important in postmenopausal women, especially because oestradiol is a more potent oestrogen than oestrone in most bioassays.

During the oestrone infusion in this study, the plasma oestrone concentrations showed marked fluctuations which presumably resulted either from episodic clearance or from episodic deconjugation of oestrone sulphate formed from oestrone during the infusion. The finding of fluctuations in the plasma oestrone concentration during both the oestrone and oestradiol infusions is consistent with the results of isotopic studies in postmenopausal women by Hembree et al. (1969) who suggested that 'non-steady state metabolic processes play a major role in oestrone clearance'. Pearlman et al. (1969) found that when isotopically labelled oestrone was infused, a steady state was not achieved and, furthermore, a large fraction of the infused oestrone was quickly conjugated, presumably in the liver. In the present study, the subjects were

not fasted and their posture was not strictly standardised and so, the episodic fluctuations in the plasma oestrone levels may have resulted from variations in hepatic blood flow and thus variations in splanchnic clearance. The fluctuations in plasma androstenedione levels that occurred during the constant infusion of androstenedione and cortisol (Subject No. 27 - Figure 2.33.) were presumably also due to variations in hepatic clearance that perhaps occurred because fluids were allowed during the infusion. However, it is uncertain at present why Longcope and Tait (1971), unlike Hembree et al. (1969) and Pearlman et al. (1969), were able to achieve a steady state during infusions of oestradiol or oestrone. The subjects studied by Longcope and Tait (1971) were, however, young and their posture was strictly standardised throughout the infusion whereas those studied by Hembree et al. (1969) and Pearlman et al. (1969) were postmenopausal, had carcinoma and their posture may not have been kept constant (Longcope and Tait, 1971).

The fluctuations in the oestradiol concentrations during the oestrogen infusions in the present study were less obvious than those of oestrone, a finding which may be consistent with the observation by Pearlman et al. (1969) of less conjugation of infused oestradiol than that of infused oestrone. However, if the fluctuations in plasma oestrone and oestradiol in normal postmenopausal women (Part 2 a) were due to episodic clearance (and not to episodic production or release from sites of production), then there must be undefined factors other than blood flow regulating their clearance because fluctuations in the plasma oestrogen concentrations were asynchronous.

d) Peripheral 'Stores': The experimental technique of observing the rate of depletion of plasma steroids after dexamethasone treatment, and the changes in plasma oestrogen levels after steroid infusions is a crude method for studying peripheral 'stores' of plasma oestrogens after the menopause, especially as the administration of glucocorticoids per se may affect the metabolic clearance rate of oestradiol (Hembree et al., 1969). The evidence that accumulated about peripheral 'stores' in this study was thus indirect and further studies are necessary to determine more exactly the storage role of the periphery that has been suggested from the results of isotopic studies. However, after the infusion of androstenedione, testosterone or ACTH, plasma levels of oestrogens, particularly oestrone, often remained elevated for at least four hours whereas this did not occur after infusions of oestradiol or oestrone (Figures 2.47.& 2.49.). This finding thus suggests that there is release of plasma oestrogens, particularly oestrone from the aromatising tissues for some considerable time after completion of the infusions. The results also showed, however, that there were considerable variations within individuals in the rate of release of oestrogens from the aromatising tissues into the circulation. Prolonged release of plasma oestrogens from the aromatising tissues would also account for the observation in this study of plasma oestrogen levels that were not significantly lowered by a single dose of dexamethasone (Table 2.7.) although this did occur after dexamethasone treatment for one week. It is also possible that the fluctuations in the plasma oestrogen concentrations in this study resulted from episodic release of oestrogen from the aromatising tissues.

As discussed earlier (Part 2 a), episodic interconversion of plasma oestrone and oestradiol with oestrone sulphate, may account for the fluctuations in the plasma levels of the unconjugated oestrogens. Hembree et al. (1969) considered that their inability to achieve a steady-state during infusions of isotopically-labelled oestrogens may have resulted from the slow re-entry into the plasma of oestrogens, particularly oestrone, from circulating conjugates. Certainly oestrone sulphate is quantitatively the dominant circulating oestrogen in postmenopausal women (Hawkins and Oakey, 1974; Anderson et al., 1978) and has a longer half-life than unconjugated plasma oestrogens (Longcope, 1972; Ruder et al., 1972) with which there is significant peripheral interconversion (Longcope, 1972). Furthermore, isotopic studies by Vaughan et al. (1976) have shown that there is rapid and significant conjugation of plasma oestradiol and oestrone in postmenopausal women with cancer. However, the results of the present study suggest that it is unlikely that oestrone sulphate will be found to be a significant intravascular 'store' of unconjugated plasma oestrogens in postmenopausal women because plasma oestrogen levels fell back to their pre-infusion levels within four hours of the completion of the infusions of oestradiol or oestrone (Figure 2.48, 2.49.). This fall in the oestrogen levels after the oestrogen infusions is consistent with the results of isotopic studies on the clearance and disappearance of plasma oestrogens (Longcope et al., 1968; Longcope and Tait, 1971; Longcope, 1972; Longcope and Williams, 1974).

In 1967, Twombley et al. showed that plasma oestradiol and oestrone could accumulate in adipose tissue. However it is unlikely that such fat 'stores' are a major source of plasma oestrogens after

the menopause because the plasma oestrogen levels fell back to their pre-infusion levels within four hours of the completion of the infusions of oestrone or oestradiol. Further studies are, however, necessary to elucidate the exact roles of adipose tissue and oestrogen conjugates in the production of plasma oestrogens after the menopause.

The falls in the plasma levels after the testosterone infusions (Figure 2.48.) in the women in this study were consistent with the clearance rates of testosterone determined in isotopic studies by Horton and Tait (1966). However, the plasma levels of testosterone were not significantly altered by a single dose of dexamethasone (Figure 2.46.)and usually remained elevated hours after infusions of ACTH or androstenedione (Figure 2.47.). Thus, the tissues in which androstenedione is converted to testosterone may be a peripheral source of testosterone for some time after it takes up the androstenedione. Androgen conjugates may, however, have a role, as yet undefined, in the production of plasma testosterone after the menopause.

PART 3

Clinical Studies

Summary: The purpose of undertaking these clinical studies of plasma oestrogens after the menopause has been described fully in Part 1. The development of superficial dyspareunia and flushes in postmenopausal women has not been related to a deficiency of any plasma oestrogen, although oestrogen therapy often relieves these climacteric symptoms. The administration of oestrogen therapy is however associated with side effects such as endometrial hyperplasia (Whitehead et al., 1977; Sturdee et al., 1978) which might, perhaps, result from oestrogen overdosage. In the present investigation the relationship of plasma oestrogens and clinical effects attributed to oestrogen deficiency, particularly vaginal atrophy, and the climacteric symptoms of superficial dyspareunia and flushes has been explored. In addition the effects of the administration of oestrogens on the plasma oestrogen and gonadotrophin concentrations of postmenopausal women were examined. These clinical studies were undertaken in the light of knowledge obtained from physiological studies (including those described in Part 2) of plasma oestrogens in postmenopausal women during their climacteric when the symptoms described above occur.

Part 3 a) RELATIONSHIP OF PLASMA OESTROGENS AND CLINICAL EFFECTS ATTRIBUTED TO OESTROGEN DEFICIENCY

As described fully in Part 1, women may suffer many symptoms during their climacteric, but only superficial dyspareunia and hot flushes and sweats are directly related to the loss of oestrogen production that results from ovarian failure or ovariectomy (Utian, 1972). Superficial dyspareunia may be a consequence of atrophy of the lower genital tract (Jeffcoate, 1975), but the pathophysiology of hot flushes and sweats is not understood (Aksel et al., 1976). Both these symptoms respond to treatment with oestrogens, although in the case of flushes the specificity of the response is contested (Mulley and Mitchell, 1976). The optimum preparation and dose of oestrogen for the treatment of women with climacteric symptoms is unknown, largely because the precise relationship of climacteric symptoms to oestrogen production in postmenopausal women is unknown.

One aim of the present investigation was to determine the exact deficiency of plasma oestrogens in women with climacteric symptoms by comparing mean oestrogen concentrations in women with superficial dyspareunia and flushes with those in asymptomatic women of similar age and menopausal status. The hormone profile studies described in Part 2 a showed that the plasma levels of oestradiol and oestrone in postmenopausal and ovariectomised women fluctuated and, in the present study, the relationship of these episodic fluctuations to the timing of flushes was also determined. Finally, this study examined the relationship of the plasma oestrogens and the karyopyknotic index of a lateral vaginal wall smear in another group of postmenopausal women.

METHOD

i) Climacteric Symptoms and Mean Oestrogen Concentrations

Twenty-five sexually active postmenopausal or ovariectomised women were studied by determining mean plasma oestrone and oestradiol concentrations in blood samples obtained every 20-30 minutes for 4-24 hours. The fifteen postmenopausal women in the group had had amenorrhoea for at least 0.4 years, had signs of atrophy of the lower genital tract (Hammond, 1976) and had plasma gonadotrophin concentrations that were in the range that is diagnostic of primary ovarian failure (Jacobs and Murray, 1976). None of the women had taken any drugs or hormone preparations for at least four months prior to the study except Subject No. 47 who was receiving l-thyroxine (300 µg/day) for primary hypothyroidism diagnosed fifteen years previously. The clinical details of each woman are summarised in Table 3.1. and described fully in Appendix IV.

Superficial dyspareunia was diagnosed if the women admitted to dryness and superficial discomfort during intercourse in the month preceding the study. The women were classified as having flushes if, in the previous month, they had experienced episodic hot feelings of any part of the body with flushing and/or sweating of the skin. Eight women had both superficial dyspareunia and flushes, a second group of eight women had flushes but no superficial dyspareunia, and the third group of nine women denied both superficial dyspareunia and flushes. No differences were detected between these groups of women with respect to their age, weight or years since the menopause, or ovariectomy (Table 3.2.). The severity of the genital tract atrophy, and the flushing

Table 3.1. Climacteric symptoms and mean ± SD oestrogen (E_1 & E_2) concentrations (ng/dl) in 15 postmenopausal and ten ovariectomised women.

NO	SUP. DYS.	FLUSHES	E_2	E_1	
1	yes	yes	2.1 ± 0.9	6.1 ± 1.2	
2	yes	yes	2.5 ± 0.9	4.3 ± 1.3	
3	no	no	3.4 ± 1.4	4.6 ± 2.0	
4	no	no	5.5 ± 1.2	7.3 ± 2.0	
5	yes	yes	1.7 ± 1.6	3.0 ± 2.0	
6	no	no	4.6 ± 0.9	6.2 ± 1.5	
7	no	yes	3.5 ± 0.9	4.7 ± 1.0	
8	no	yes	3.8 ± 1.4	7.3 ± 3.5	Postmenopausal
9	yes	yes	1.7 ± 1.0	7.0 ± 1.6	
12	yes	yes	3.3 ± 1.4	5.4 ± 0.4	
13	no	yes	2.8 ± 0.7	3.9 ± 1.6	
14	yes	yes	2.2 ± 1.0	3.7 ± 1.0	
15	no	no	2.9 ± 1.7	5.5 ± 0.6	
16	no	no	5.4 ± 1.0	5.6 ± 2.6	
36	no	yes	5.5 ± 0.6	4.3 ± 1.0	
17	no	yes	4.0 ± 1.4	6.4 ± 3.2	
18	no	yes	4.9 ± 1.0	4.6 ± 1.0	
19	no	yes	2.7 ± 0.9	4.9 ± 1.0	
20	yes	yes	2.3 ± 0.4	3.2 ± 1.1	
21	no	no	3.1 ± 0.9	8.2 ± 0.7	Ovariectomised
23	no	no	3.4 ± 0.9	3.0 ± 1.4	
24	no	no	3.6 ± 1.0	7.4 ± 1.1	
25	yes	yes	2.5 ± 1.1	7.0 ± 1.9	
26	no	no	2.6 ± 0.7	6.9 ± 1.5	
47	no	yes	5.6 ± 1.8	3.8 ± 2.1	

was not assessed.

ii) Fluctuations in Hormone Levels and Occurrence of Flushes

Five of the 16 women with flushes (Subject Nos. 1,2,5,8 & 17) described above were studied by obtaining blood samples every 20 minutes for 24 hours. During this time, each woman was asked to note the exact time of each flush. Blood samples were also obtained every 20 minutes for 24 hours from two of the nine asymptomatic women (Subject Nos. 3 & 4) described above. Plasma cortisol, androstenedione, testosterone, oestrone, oestradiol, FSH and LH concentrations were determined in all blood samples. The pattern of the hormone levels in the symptomatic women was compared with the pattern in the asymptomatic women. In the five symptomatic women the timing of each flush was related to changes in the plasma steroid concentrations.

iii) Plasma Oestrogen Levels and Karyopyknotic Indices

Forty-four women who consented to uterine cavity aspiration (Hutton et al., 1978) and who had had no oestrogen or progestogen therapy in the previous three months were included in this study. None had had periods in the two months prior to the examination, and their plasma gonadotrophin concentrations were raised into the range regarded as diagnostic of primary ovarian failure (Jacobs and Murray, 1976). The mean age of the women was 56.5 $\pm$ 8.9 (SD) years, (range 46-80 years), and the mean interval since their last menstrual period was 7.0 $\pm$ 9.1 years (range 0.2-34.0 years).

A lateral wall smear obtained at the time of uterine cavity aspiration was fixed in 95% alcohol and subsequently stained by the Papanicolaou-Traut method (Papanicolaou and Traut, 1943).

The karyopyknotic index (which is the ratio of superficial cells with pyknotic nuclei to intermediate cells) was determined by the cytologist Mrs A.M. Morse, from a minimum total count of 500 cells. Superficial cells in a lateral vaginal wall smear may result not only from oestrogenic stimulation but also from infection, progestogens and mechanical irritation such as that caused by prolapse. (Wachtel, 1969). However, mechanical irritation also causes an increase in the number of parabasal cells, and so the results from subjects whose smears showed a predominance of parabasal cells over superficial cells were excluded from statistical analyses.

Plasma oestrone and oestradiol concentrations were determined in duplicate in the blood samples that were obtained from all subjects after the pelvic examination.

RESULTS

i) Climacteric Symptoms and Mean Oestrogen Concentration

The mean plasma oestrone and oestradiol concentrations in each of the 25 women are detailed in Table 3.1. The mean plasma oestrogen concentrations in the three groups of women are shown in Table 3.2. The group of mean plasma oestradiol concentrations (Figure 3.1.) in the women with superficial dyspareunia and flushes was significantly lower ($P < 0.001$ - Mann Whitney U test) than the group concentrations in either the women with flushes only, or the asymptomatic women. In contrast, there was no difference in the mean plasma oestrone concentrations between the three groups of women (Figure 3.2.).

ii) Fluctuations in Hormone Levels and Flushes

The intensive hormone profiles of the five symptomatic women (Subject Nos. 1,2,5,8 & 17) are shown in Figures 2.1., 2.2., 3.3.,

Table 3.2. Mean ± SD age, years since menopause or ovariectomy, weight and plasma oestrogen concentrations in three groups of postmenopausal or ovariectomised women.

SYMPTOM GROUP	AGE	YEARS MP/OV	WEIGHT Kg	E_2 ng/dl	E_1 ng/dl
Sup. dysp. & flushes (n=8)	49.3 ± 4.9	2.6 ± 1.6	58.9 ± 6.3	2.3 ± 0.5	5.0 ± 1.6
Flushes only (n=8)	51.4 ± 3.1	4.0 ± 4.4	62.8 ± 5.0	4.1 ± 1.1	5.0 ± 1.2
Asymptomatic (n=9)	51.2 ± 2.9	4.5 ± 4.3	64.4 ± 7.1	3.8 ± 1.1	6.1 ± 1.6

Figure 3.1. Mean plasma oestradiol in postmenopausal and ovariectomised women either with superficial dyspareunia and flushes (both symptoms), with flushes only, or with neither symptom.

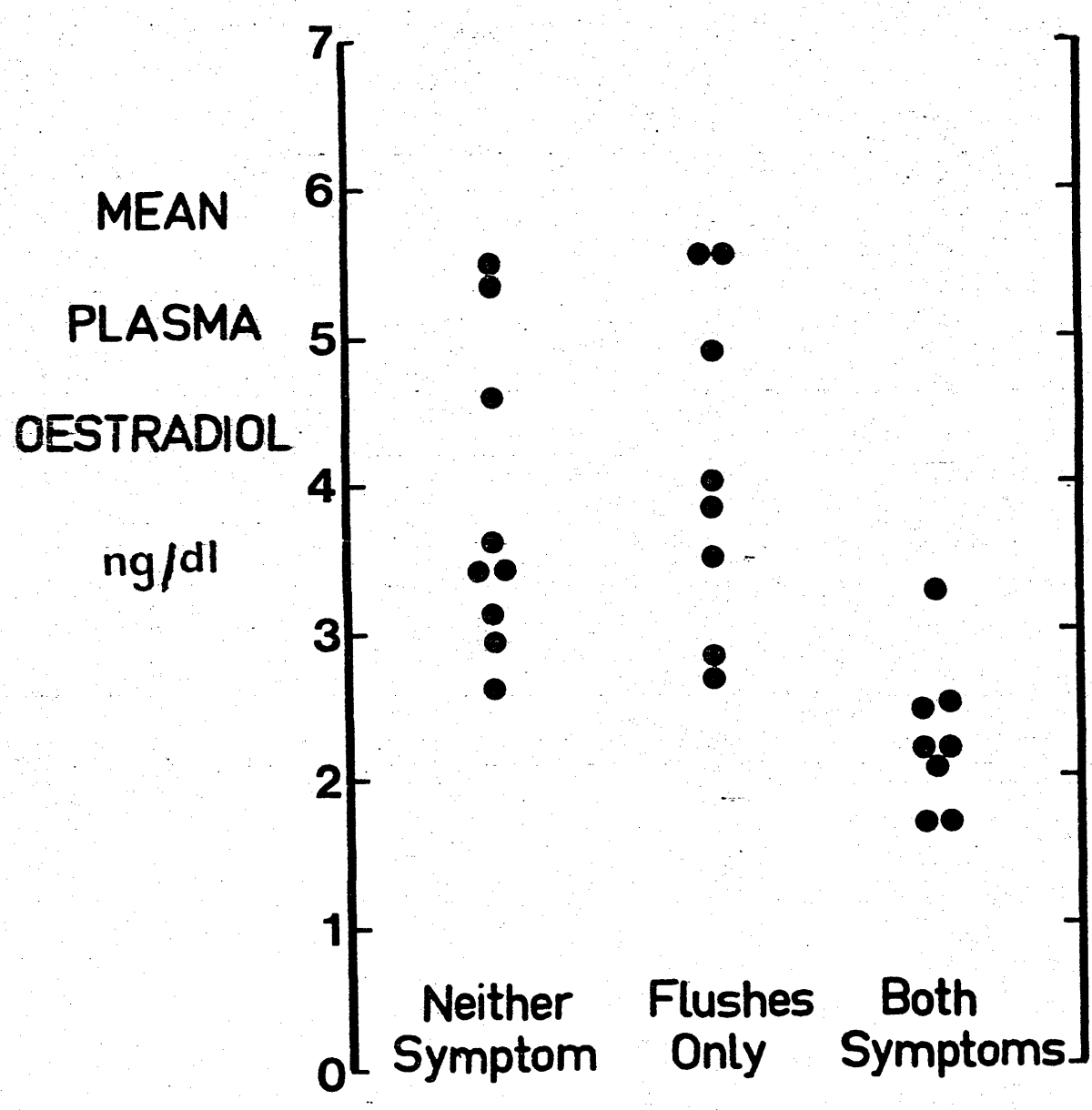

MEAN
PLASMA
OESTRADIOL
ng/dl
7
6
5
4
3
2
1
0
Neither Symptom
Flushes Only
Both Symptoms

Figure 3.2. Mean plasma oestrone concentrations in postmenopausal or ovariectomised women either with superficial dyspareunia and flushes (both symptoms), with flushes only, or with neither symptom.

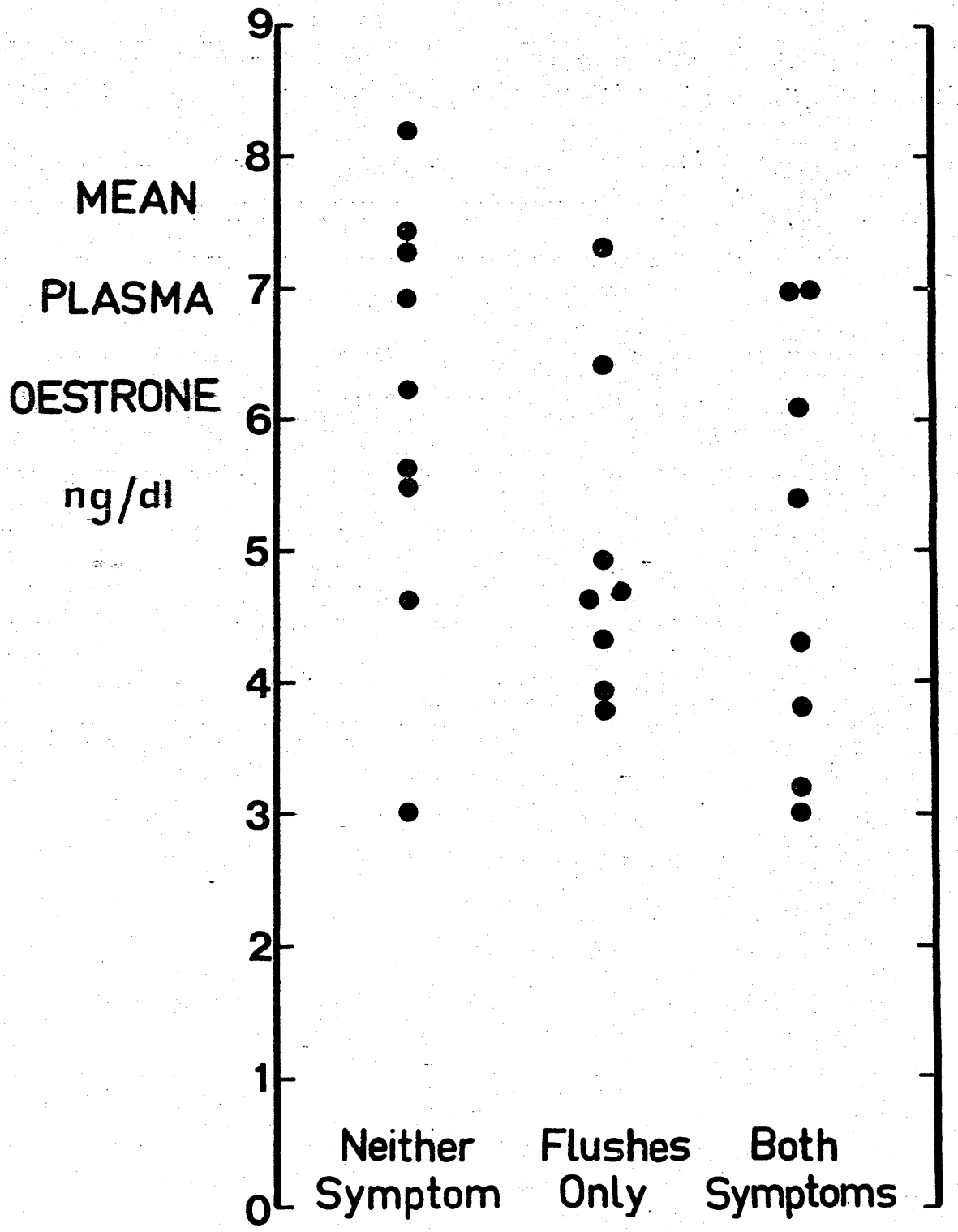

MEAN
PLASMA
OESTRONE
ng/dl
9
8
7
6
5
4
3
2
1
0
Neither Symptom
Flushes Only
Both Symptoms

Figure 3.3. Plasma testosterone, oestrone and oestradiol, FSH and LH concentrations in Subject No. 5. The time of each flush is denoted:↓

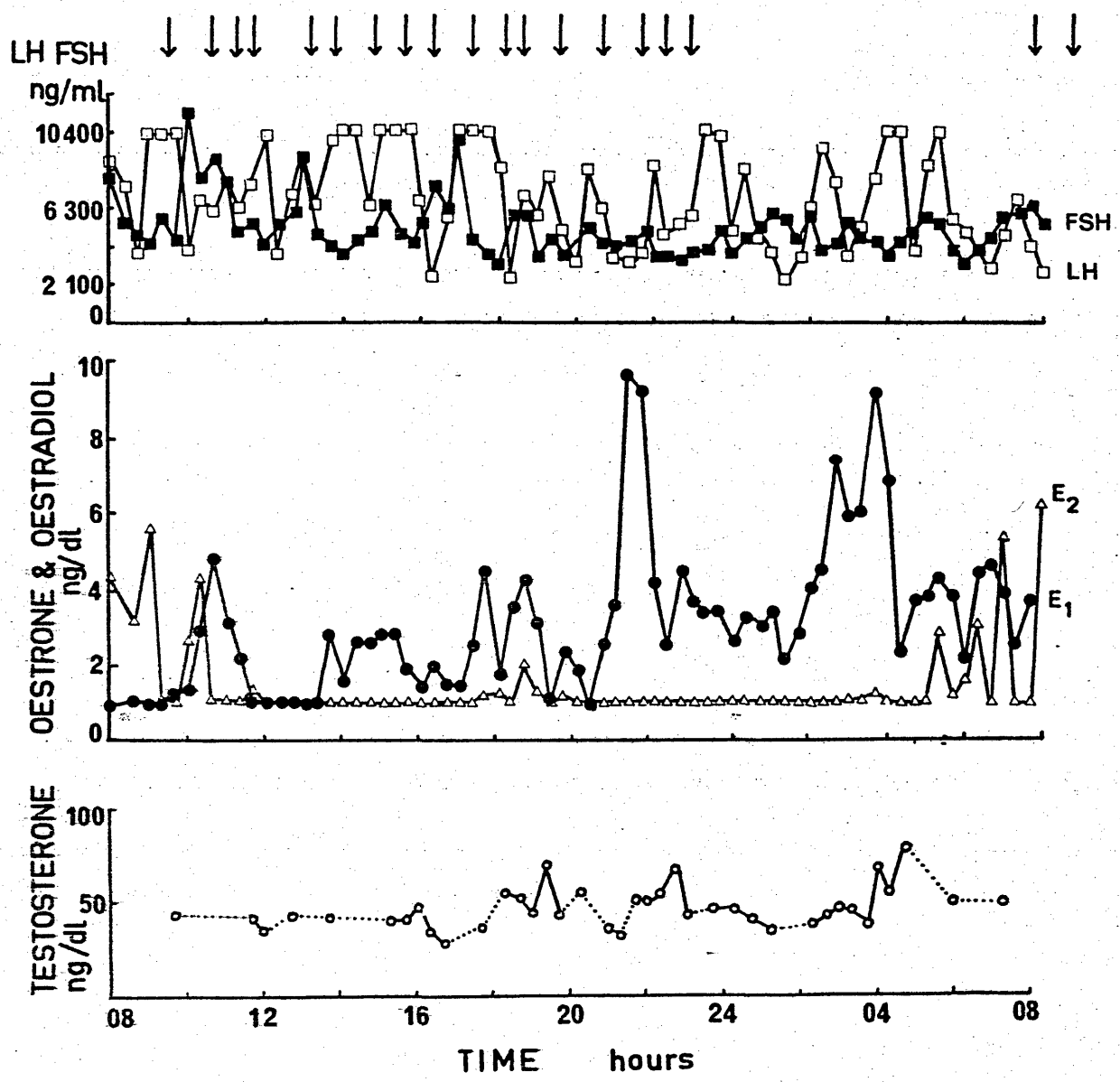
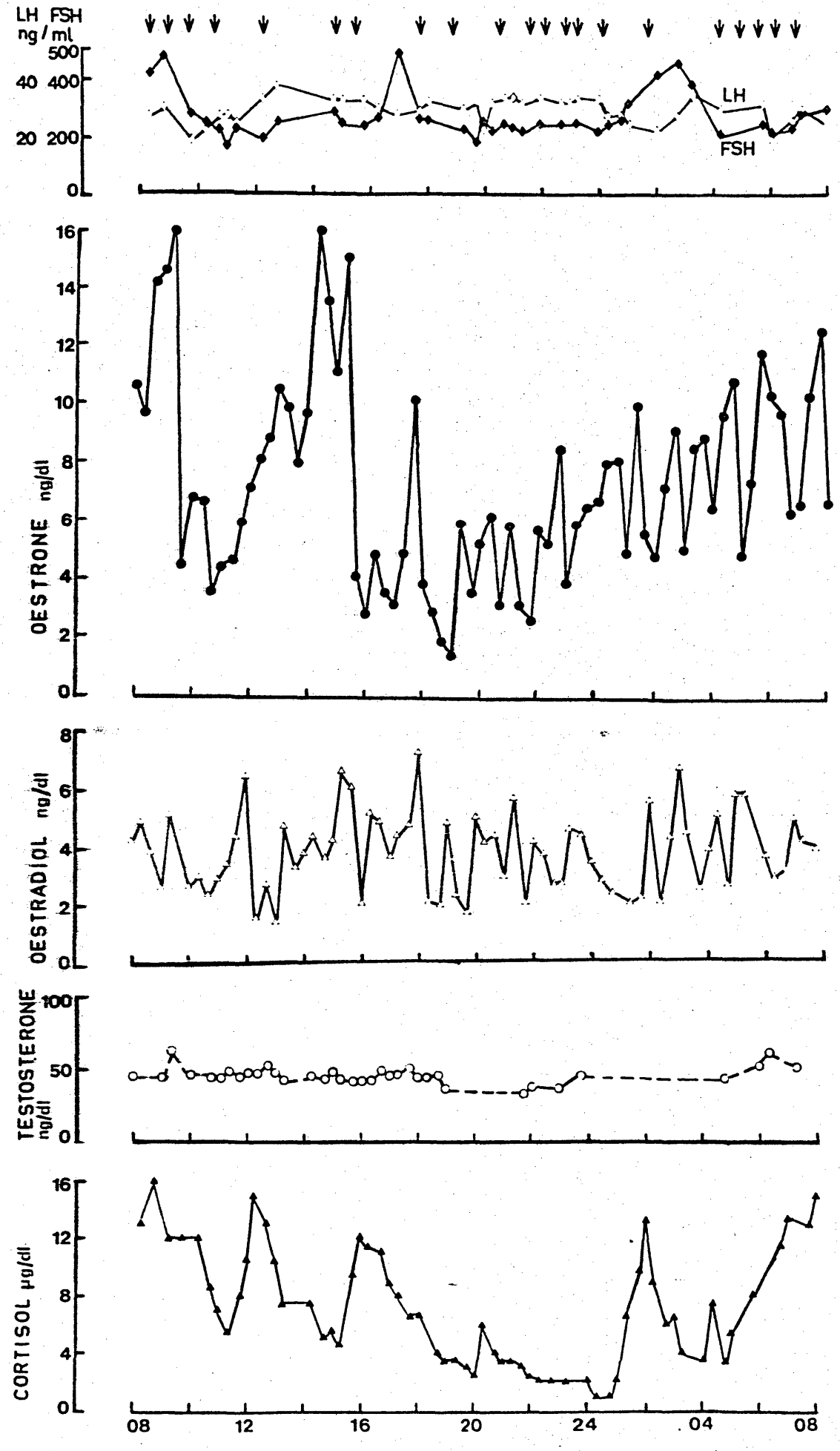

LH FSH
ng/ml
10400
6 300
2 100
0
FSH
LH
OESTRONE & OESTRADIOL
ng/dl
10
8
6
4
2
0
E2
E1
TESTOSTERONE
ng/dl
100
50
08
12
16
20
24
04
08
TIME hours

Figure 3.4. Plasma steroid and gonadotrophin concentrations in Subject No. 8. The time of each flush is denoted ↓


LH FSH
ng / ml
500
40 400
20 200
0
LH
FSH
16
14
12
10
8
6
4
2
0
OESTRONE ng/dl
8
6
4
2
0
OESTRADIOL ng/dl
100
50
0
TESTOSTERONE ng/dl
16
12
8
4
0
CORTISOL µg/dl
08
12
16
20
24
04
08

3.4. & 2.13. respectively. The time of each flush experienced by the symptomatic women is also shown. There was no obvious relation between the timing of a flush and a particular hormone level or to a rise or fall in the concentration. In addition the pattern of the hormone levels in the profiles of the women with flushes appeared similar to those in the profiles of the asymptomatic women (Figures 2.3. & 2.4.).

iii) Plasma Oestrogen Levels and Karyopyknotic Indices

The lateral vaginal wall smear was too scanty for a reliable assessment in three women (two of whom had procidentia) and so a karyopyknotic index was determined only in 41 women. However, the results of three of these women, who all had second or third degree prolapse, were excluded from the statistical analyses because in their smears, parabasal cells predominated over superficial cells. The karyopyknotic index in each of these women was 7%, 14% and 35%, and their respective plasma oestrogen concentrations were 4.0 ng/dl, 4.0 ng/dl and 3.2 ng/dl for oestradiol and 7.9 ng/dl, 6.8 ng/dl and 6.0 ng/dl for oestrone.

The karyopyknotic index, and plasma oestradiol and oestrone concentrations for each of the 38 women included in the statistical analysis are shown in Table 3.3. The correlations of the karyopyknotic index with the plasma oestradiol and oestrone concentrations in these women are shown in Figures 3.5. and 3.6. respectively. A highly significant correlation ($\rho = 0.581$, $P < 0.001$ - Spearman's Rank Correlation Coefficient test) was found between the karyopyknotic index and the concentration of plasma oestradiol. There was no significant correlation of the plasma oestrone concentration with

Table 3.3. Clinical details, karyopyknotic index (KPI) and random oestrogen (E_1 & E_2, ng/dl) concentrations in 39 women with biochemical evidence suggestive of primary ovarian failure. (years' denotes years since last menstrual period)

NO	AGE	YEARS	HT (cm)	WT (Kg)	KPI %	E_2	E_1
H1	56	4.0	163	61.6	0	2.9	5.4
H2	52	0.5	163	60.3	6	5.5	12.5
H3	63	10.0	160	76.2	0	2.7	6.7
H7	55	6.0	170	79.3	0	3.2	13.2
H9	57	7.0	164	56.2	0	3.4	20.8
H10	55	3.0	164	54.9	0	3.6	16.9
H11	51	1.1	152	64.4	5	3.0	7.5
H12	47	0.2	159	47.6	4	4.5	9.6
H13	51	0.4	151	50.8	19	5.6	18.6
H17	61	10.0	150	50.8	0	3.7	14.1
H21	46	5.0	157	58.0	14	3.5	11.0
H22	66	13.0	152	40.8	4	3.9	14.9
H28	50	1.7	168	57.1	12	3.7	19.7
H31	62	15.0	157	59.8	0	3.7	4.7
H35	46	0.5	166	60.3	16	3.3	6.5
H45	48	0.2	-	-	7	3.4	9.8
H46	64	13.0	160	54.9	0	2.9	9.5
H53	69	11.0	165	69.8	0	3.1	6.2
H56	47	0.4	168	88.9	4	3.1	9.6
H57	53	3.0	157	73.0	6	5.2	8.2
H59	63	13.0	168	63.5	14	5.0	16.8
H62	49	0.2	152	58.5	6	3.6	7.5
H66	74	25.0	163	59.0	16	3.2	10.2
H79	67	15.0	163	60.3	9	3.0	2.8
H71	53	0.3	157	63.5	0	2.6	7.8
H72	46	1.2	163	56.2	53	7.5	5.9
H75	56	0.7	164	75.3	4	6.7	1.0
H76	52	1.0	155	59.0	10	5.7	1.0
H82	65	0.3	165	76.2	16	7.1	13.4
H83	68	24.0	163	52.6	0	3.8	8.9
H85	80	34.0	160	63.5	4	3.5	11.5
H89	46	0.4	168	102.0	33	4.7	11.8
H93	54	0.2	163	76.2	0	3.0	4.8
H94	49	0.4	155	50.8	2	3.5	1.3
H95	72	33.0	152	57.1	12	4.7	3.0
H96	50	7.0	157	54.0	0	3.9	3.2
H97	51	0.4	155	52.6	0	3.0	6.1
H98	52	5.0	171	72.5	0	2.4	5.4

Figure 3.5. Correlation of karyopyknotic indices (KPI) and random plasma oestradiol concentrations in 38 women with biochemical evidence of primary ovarian failure. (Spearmans Rank Correlation coefficient test (ρ) was employed to determine significance)

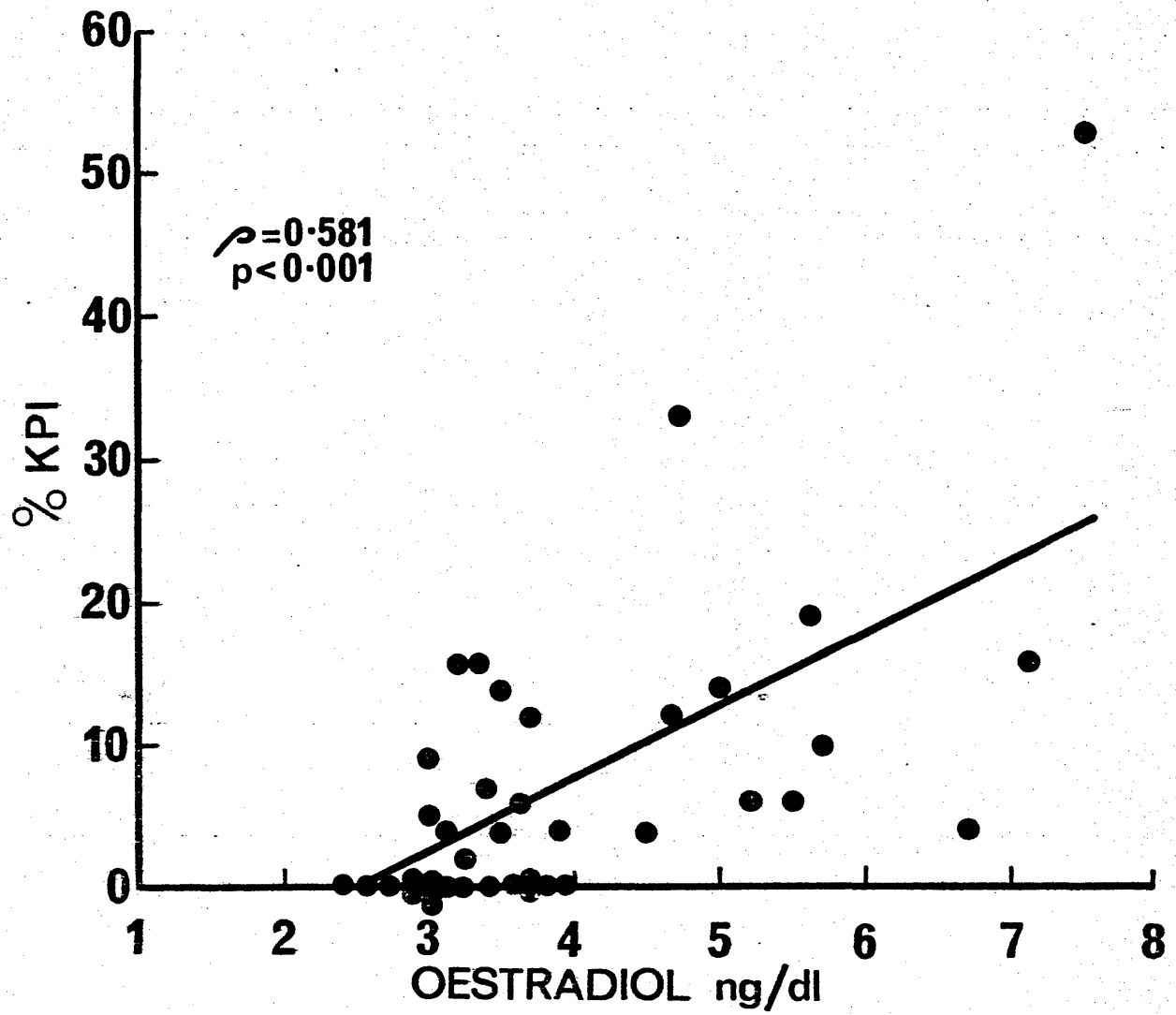
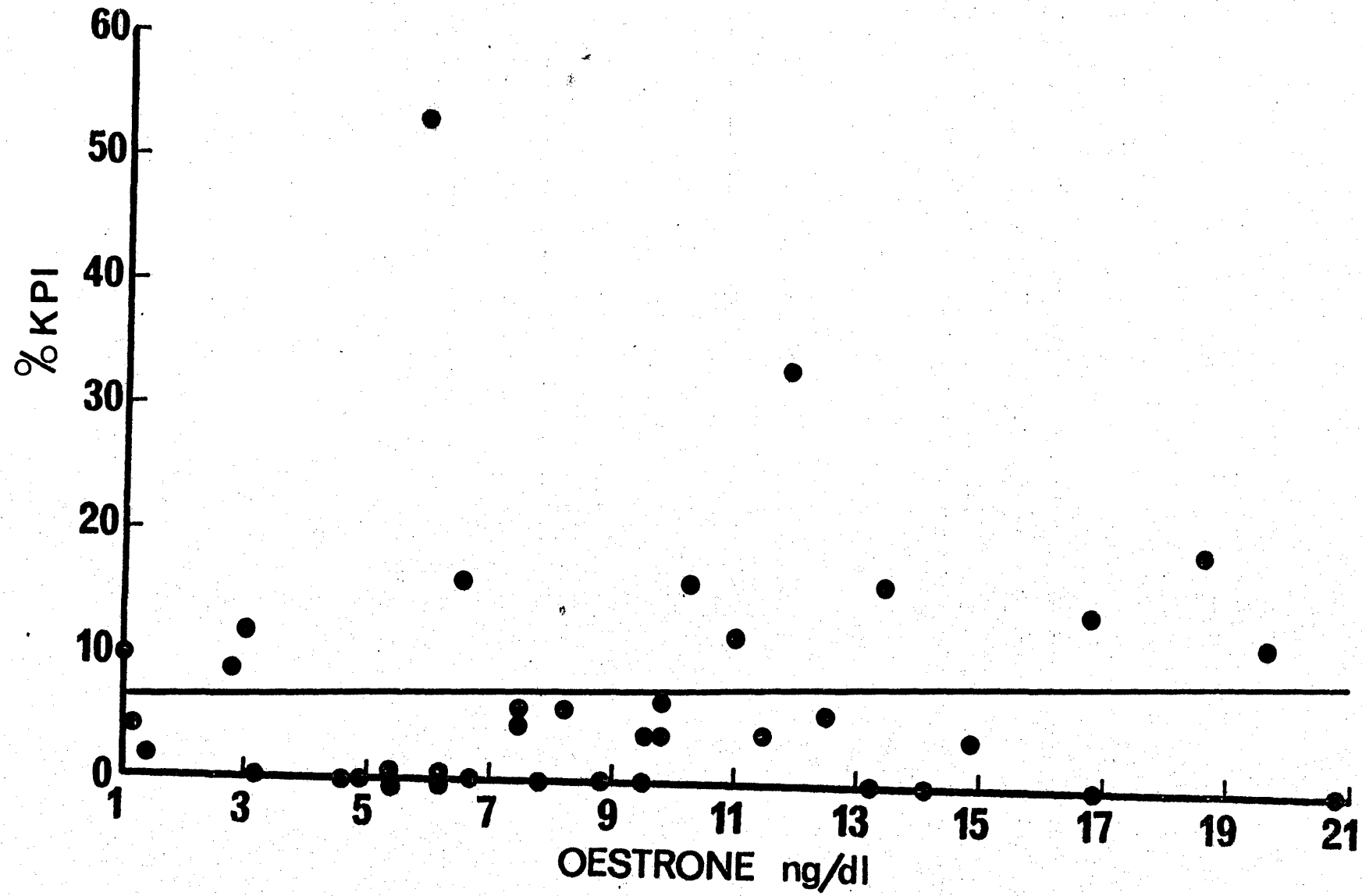

60
50
40
30
20
10
0
% KPI
ρ=0·581
p<0·001
1
2
3
4
5
6
7
8
OESTRADIOL ng/dl

Figure 3.6. Correlation of karyopyknotic indices (KPI) and random plasma oestrone concentrations in 38 women with biochemical evidence of primary ovarian failure. (Spearmans Rank Correlation coefficient test (ρ) was employed to determine significance).


60
50
40
30
20
10
0
%KPI
1
3
5
7
9
11
13
15
17
19
21
OESTRONE ng/dl

the karyopyknotic index ($\rho = 0.194$).

DISCUSSION

In this study, the mean plasma oestradiol concentrations in the postmenopausal and ovariectomised women who had both superficial dyspareunia and flushes were significantly lower than the levels in either the asymptomatic women, or the women with flushes only. Although no women in this study had superficial dyspareunia and no flushes (presumably because only women during the climacteric were studied) superficial dyspareunia was clearly the symptom that was associated with low mean plasma oestradiol concentrations. The complaint of dyspareunia may, therefore, be attributed to a more severe degree of vaginal atrophy than occurred in the asymptomatic women. This hypothesis was supported by the finding of a positive correlation between random plasma oestradiol concentrations and the karyopyknotic indices (Figure 3.5.).

The karyopyknotic index is a popular method of determining cytologically the degree of oestrogenic proliferation of the vaginal epithelium, although other factors such as prolapse, progestogens and infection may also affect the index (Wachtel, 1969; Hammond, 1977). The results of the present study augment the findings of a positive correlation of the karyopyknotic index and random plasma oestradiol concentrations in a group of pre- and postmenopausal women studied by Schneider et al. (1977). However in this latter study, the sensitivity of the oestradiol radioimmunoassay was only 4 ng/dl, and the plasma oestradiol concentrations were only measurable in the premenopausal women. Furthermore, no allowance was made for progesterone secreted

during the luteal phase of the cycle antagonising oestrogen-induced proliferation of the vaginal epithelium. Although in the present study, progesterone estimations were not made, ovarian secretion of progesterone was considered unlikely as nearly all the women were postmenopausal. Ovarian secretion was, however, likely in those few women whose plasma oestradiol concentrations were 5 ng/dl even though their gonadotrophin concentrations were raised.

In postmenopausal and ovariectomised women, plasma oestradiol concentrations fluctuate throughout the day (as shown in Figures 3.3. & 3.4 and discussed in Part 2 a). The wide scatter of the plasma oestradiol concentrations about the regression line may therefore have been due to the inaccuracy of a concentration determined in a single blood sample in reflecting the mean oestradiol concentration in blood samples obtained over a longer period of time (Part 2 a). Obviously further studies are necessary to determine the accuracy with which the karyopyknotic index reflects the plasma oestrogen status of the postmenopausal woman, but the results of this study do suggest that this simple cytological method may become a valuable aid in the management of postmenopausal women, especially those prescribed oestrogen therapy. The karyopyknotic index may, however, be influenced by conditions such as prolapse or infection although in this study, women in whom these disorders may have affected the index were excluded cytologically when parabasal cells predominated over superficial cells. Although the maturation value (Meisels, 1967) is a differential count of the superficial, intermediate and parabasal cells, the determination of the karyopyknotic index with a qualitative assessment of the parabasal cells when superficial cells occur

may be a simpler and more reliable method of estimating oestrogen status. This is especially so as Schneider et al. (1977) found that, unlike the karyopyknotic index, there was no significant correlation between the maturation value and the plasma oestradiol concentration.

This study showed that the occurrence of flushes is not related to a particular concentration, mean level or pattern of plasma oestradiol or oestrone, or to a change in the levels of any of the other hormones. Campbell et al. (1976) suggested, in a study in which blood samples were obtained every two hours, that the timing of a flush may be related to changes in plasma androstenedione levels, but this was not apparent in this study where samples were obtained every 20-30 minutes. Moreover, although there was a diurnal secretion of androstenedione, there was no such diurnal rhythm in the frequency of the flushes. Recent physiological studies by Sturdee et al. (1978) have suggested that the onset of the hot flush is associated with a sudden and transient increase in sympathetic drive. The autonomic nervous system is under the influence of several stimuli including hormones such as oestrogens and gonadotrophins. However, further studies of the role of alterations in the plasma oestrogen and gonadotrophin concentrations that occur during the climacteric in the pathogenesis of flushes are necessary so that more rational management of women with this distressing symptom can be developed.

The results of this study support the concept that, at all stages of life, oestradiol is biologically the most potent circulating oestrogen. In contrast, the physiological role of plasma oestrone in postmenopausal women remains to be determined, especially as there was no obvious relation between plasma

oestrone and either climacteric symptom in the present study.

One concept of 'hormone replacement therapy' is the prescription of oestrogens to repair a deficiency of plasma oestrogens in symptomatic postmenopausal women. The results of this study show that the accurate fulfil ment of this concept requires such therapy to increase the plasma oestradiol, but not oestrone, concentrations in women with superficial dyspareunia, to levels about 3.8 ng/dl. However, when current dosages of pure natural oestrogens are administered orally to postmenopausal women as 'hormone replacement therapy' (Part 3 b), the plasma concentrations of oestradiol often exceed those found in the asymptomatic women in this study. Furthermore, the plasma oestrone levels during treatment in women who require this therapy (Part 3 b) are always very much higher than the levels observed in the postmenopausal women in this study. Further studies are required to determine the exact significance of these unphysiological levels of plasma oestrone.

Part 3 b) EFFECT OF POSTMENOPAUSAL OESTROGEN THERAPY ON PLASMA OESTROGEN AND GONADOTROPHIN CONCENTRATIONS

Many commercial preparations are now available to treat postmenopausal women. However, the effects on plasma oestrogens of the administration of piperazine oestrone sulphate or oestradiol valerate can be studied because the absorbed oestrogens complement the endogenous circulating oestrone and oestradiol. One aim of this study was to determine the accuracy with which these preparations fulfil the biochemical concepts detailed in Part 1 of 'hormone replacement therapy' by measuring plasma oestrone and oestradiol concentrations in women throughout either the first day of therapy. or the last day of the treatment cycle.

Yen et al. (1975) showed that the oral administration of oestradiol preferentially increased the plasma oestrone concentrations whereas Rigg et al. (1977) found that the intravaginal administration preferentially increased the oestradiol fraction of plasma oestrogens. A further aim of this study was to compare the effects on plasma oestrone and oestradiol of the oral and parenteral routes of oestrogen administration.

Studies by Nillius and Wide (1970) and Tsai and Yen (1971), and Yen et al. (1975). showed that there was a differential release of FSH and LH after the administration of oestrogens to postmenopausal women. More recently, Sherman and Korenman (1975), Sherman et al. (1976) and Van Look et al. (1977) have found that FSH but not LH concentrations were raised in some premenopausal women during the climacteric. Another aim of this study was to examine the effect of changes in the plasma oestrogen concentrations on the differential release of FSH and LH in postmenopausal and

ovariectomised women.

METHOD

i) Effects on Plasma Oestrogen Concentrations

Oral: Four groups of postmenopausal or ovariectomised women were studied by determining plasma oestrone and oestradiol concentrations in blood samples obtained every 30 minutes for 24 hours, during the first four hours of which either piperazine oestrone sulphate or oestradiol valerate was administered orally.

Group I and group II consisted of six women who were administered piperazine oestrone sulphate (Subject Nos. 5,9,10,24,40& 42) and oestradiol valerate (Subject Nos. 11,12,14,15,37 & 41) respectively on the first day of a three week course of cyclical therapy. Four women in each of these two groups had not had oestrogen therapy , whilst the other two women in these groups were studied after the treatment-free week. In groups I and II four of the six women were prescribed the lower dose of oestrogen (i.e. 1.5 mg of piperazine oestrone sulphate or 1 mg of oestradiol valerate) whilst two of the women were prescribed the higher dose (i.e. 3.0 mg of piperazine oestrone sulphate or 2 mg of oestradiol valerate). The oestrogen therapy was prescribed primarily for the relief of flushes except in two women (Subject Nos. 24 in group I & Subject No. 15 in group II) in whom therapy was prescribed for depression or impaired libido. Flushes were present at the time of study in four women in each group, but during the three weeks of treatment subsequent to the study, the flushes resolved in four women (Subject Nos. 9,10 & 42 in group I and Subject No. 14 in group II). In these two groups, one subject (No. 40 in group I) had vaginal bleeding during the treatment free

week and, incidentally, also had breakthrough bleeding. Endometrial cells aspirated at the time of study showed the features of normal proliferation (Hutton et al., 1978). A vaginal examination was not always performed at the time of the study but this incidental examination in Subject No. 42 (group I) did not show, unlike other examinations, the clinical features of epithelial atrophy (Hammond, 1976) which classically occur in postmenopausal women.

Groups III and IV consisted each of three women who were administered piperazine oestrone sulphate (3 mg) (Subject Nos. 13, 45 & 46) and oestradiol valerate (2 mg) (Subject Nos. 38,43 & 44) respectively and were studied on day 17-21 of the cyclical therapy. These women all took their oestrogen at the same time each day, including the day of the study. The therapy was being prescribed for flushes, and was affording relief in all women except No. 43 (group IV). One woman (Subject No. 13 in group III) had withdrawal bleeding during the treatment-free week and endometrial cells aspirated two months after the study showed the cytological features of cystic hyperplasia (Hutton et al., 1978). Bleeding in one other woman (Subject No. 38, group IV) was irregular and endometrium obtained at curettage the day after the study showed the histological features of endometrial hyperplasia (Anderson, 1978). Three weeks later a hysterectomy and bilateral ovariectomy was performed because of fibroids and the hyperplasia. No primordial follicles were seen histologically in the ovary (Anderson, 1978).

The clinical details of each woman studied are summarised in Table 3.4. and given in full in Appendix IV. The gonadotrophin concentrations of all women were raised into the range that was

Table 3.4. Clinical details of 18 postmenopausal or ovariectomised (Ov) women studied on either the first day or towards the end of the treatment cycle of either piperazine oestrone sulphate (PE_1S) or oestradiol valerate (E_2V).

	NO	AGE YRS	YEARS MP/OV	WT KG	PREV. YRS. THER.	OTHER REL. DETAILS	CLIMACT. SYMP. FLUSHES	CLIMACT. SYMP. SUP. DYS.	RESP. TO THERAPY IMPROVE.	RESP. TO THERAPY WITH. BLEED	TYPE	OESTROGEN DOSE	DAY OF COURSE
	24	49	$6.0^{Ov.}$	60	Nil	Depress.	No	No	-	-	PE_1S	1.5	1
	42	48	0.9	67	Nil	-	Yes	Yes	Yes	No	PE_1S	1.5	1
GP. I	5	55	4.5	54	0.5	-	Yes	Yes	No	No	PE_1S	1.5	1
	40	50	3.0	64	2.8	-	No	No	-	Yes	PE_1S	1.5	1
	9	48	0.4	56	Nil	-	Yes	Yes	Yes	No	PE_1S	3.0	1
———	10	62	8.0	70	Nil	-	Yes	-	Yes	No	PE_1S	3.0	1
	11	51	2.6	51	Nil	-	Yes	-	No	No	E_2V	1.0	1
	12	51	0.9	57	Nil	-	Yes	Yes	No	No	E_2V	1.0	1
Gp.	15	49	2.1	56	Nil	Imp. Libido	No	No	-	No	E_2V	1.0	1
II.	37	33	$2.0^{Ov.}$	51	0.5	-	Yes	Yes	No	-	E_2V	1.0	1
	14	51	1.6	58.5	Nil	-	Yes	Yes	Yes	No	E_2V	2.0	1
———	41	51	2.0	65	0.5	-	No	No	-	No	E_2V	2.0	1
	13	54	3.0	60	0.5	-	No	No	-	Yes	PE_1S	3.0	21
GP. III	45	54	2.2	54	2.0	-	No	No	-	No	PE_1S	3.0	20
———	46	50	1.1	61	0.6	-	No	No	-	No	PE_1S	3.0	21
	38	56	3.4	57.5	1.5	Aden. Hyp.	No	No	-	Yes	E_2V	2.0	17
GP. IV	43	48	$0.6^{Ov.}$	73	0.3	-	Yes	-	No	-	E_2V	2.0	21
	44	49	1.5	57	0.9	-	No	No	-	No	E_2V	2.0	20

diagnostic of primary ovarian failure (Jacobs and Murray, 1976).

Parenteral: The effects on plasma oestrogen concentrations of the parenteral administration of oestrogens was studied in six postmenopausal or ovariectomised women. In three of these women, oestradiol (Subject Nos. 32 & 33) or oestrone (Subject No. 34) was administered intravenously after prior treatment with dexamethasone, as described in Part 2 d. In one postmenopausal woman (Subject No. 36) oestradiol benzoate (1 mg) was injected intramuscularly four hours after the commencement of a 20 hour sampling period. Plasma oestrone and oestradiol concentrations were determined in the blood samples that were obtained every 30 minutes during this time, and in two blood samples that were obtained two and four hours after the completion of the intensive sampling period. In three ovariectomised women (Subject Nos. 17 and 34), a pellet of oestradiol (25 mg) was inserted under local anaesthesia into the subcutaneous fat of the anterior abdominal wall. All these women had had ethinyl oestradiol (20 µg/day) prescribed cyclically for at least two months without relief of their flushes, but this therapy was discontinued two weeks prior to the implant. In one woman (Subject No. 17) the flushes recurred five months after the implant, and another pellet was implanted, together with a pellet of testosterone which was prescribed for loss of libido which had not responded to other forms of therapy. Blood samples were obtained before and at intervals after the implant operations.

ii) Effects on Plasma Gonadotrophin Concentrations

Oral: The effects of oral administration of a single dose

of oestrogen on the plasma gonadotrophin concentrations was investigated in four of the women described above (Subject Nos. 5,9,14 & 24). These women were studied, as described above, on the first day of a course of therapy. Plasma FSH and LH concentrations were determined in the blood samples that were obtained every 30 minutes for 24 hours. Before the ingestion of the single dose of either piperazine oestrone sulphate or oestradiol valerate, the oestrogen concentrations of these four women were within the normal range for postmenopausal women (Part 2 a).

Parenteral: Plasma FSH and LH concentrations were determined in the three women infused oestradiol or oestrone (Subject Nos. 32,33 & 34) as described above, and in Part 2 d. Plasma FSH and LH concentrations were also determined in the blood samples obtained from the woman (Subject No. 36) given the intramuscular injection of oestradiol benzoate (on 11.1.77). The chronic effect of oestrogen administration on gonadotrophin concentrations was also studied in this subject by prescribing 30 µg/day of ethinyl oestradiol cyclically and then restudying the woman at the end of the third treatment cycle (on 5.4.77) when the injection of oestradiol was also repeated to determine if there was any alteration in the gonadotrophin response to the parenteral administration of a high dose of oestrogen.

The effect on the gonadotrophin concentrations of the subcutaneous implants of oestradiol (25 mg) was studied in the two ovariectomised women (Subject Nos. 17 & 34) by determining the plasma FSH and LH concentrations in the blood samples that were obtained as described above.

RESULTS

i) Effects on Plasma Oestrogen Concentrations

Oral: The hormone profiles of the plasma oestrone and oestradiol concentrations of the six women in group I studied on the first day of a course of piperazine oestrone sulphate are shown in Figure 3.7, and those of the six women in group II prescribed oestradiol valerate in Figure 3.8. The pretreatment plasma oestrone concentrations in all women except Subject No. 42 (Figure 3.6.) were similar to those normally seen in postmenopausal or ovariectomised women (Part 2 a and 2 b). The pretreatment plasma oestradiol concentrations in four of the women in group I and five of the women in group II were also similar to those seen in postmenopausal women (Part 2 a). However, the pretreatment plasma oestradiol concentrations in two women in group I were about 9 ng/dl. One woman (Subject No. 40) had had oestrogen therapy for three years, after a four month episode of amenorrhoea and was experiencing irregular vaginal bleeding at the time of study, whilst the other woman (Subject No. 42) had ten months of amenorrhoea and flushes but did not have withdrawal bleeding nor, incidentally, atrophic vaginitis. The pretreatment plasma oestradiol concentration in Subject No. 41 (group II) was 5.4 ng/dl which is just above the upper limit of the normal postmenopausal range for women of this age.

The effects of administered piperazine oestrone sulphate and oestradiol valerate in groups I and II respectively appeared similar. After ingestion of the tablets on the first day of therapy there was usually a marked increase of plasma oestrone concentrations and the levels rose to >40 ng/dl in two of the four women given the higher dosages of the oestrogen preparations.

Figure 3.7. Plasma oestrone (X) and oestradiol (●) concentrations in six postmenopausal or ovariectomised women prescribed piperazine oestrone sulphate on the first day of a three week treatment cycle.

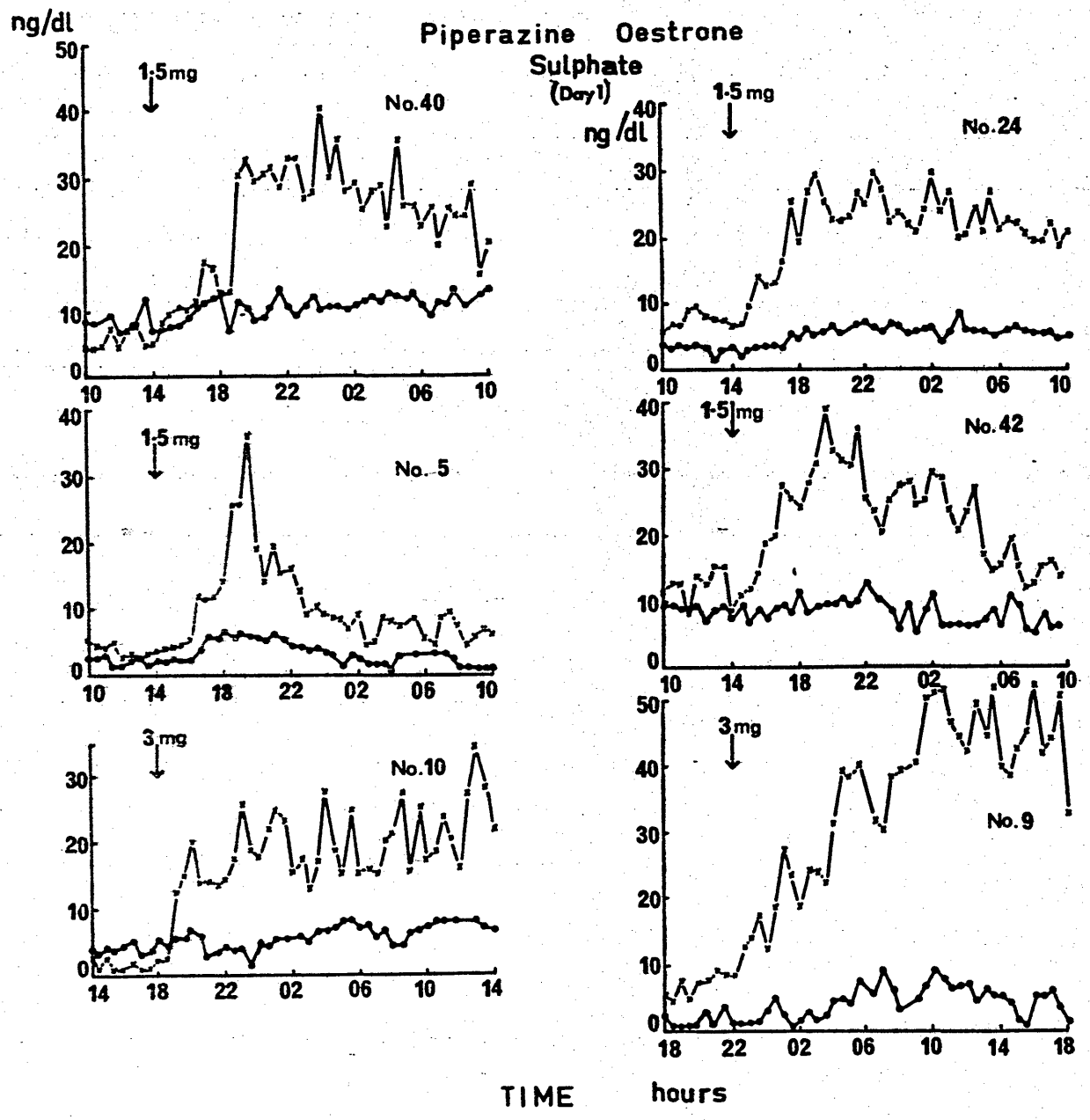
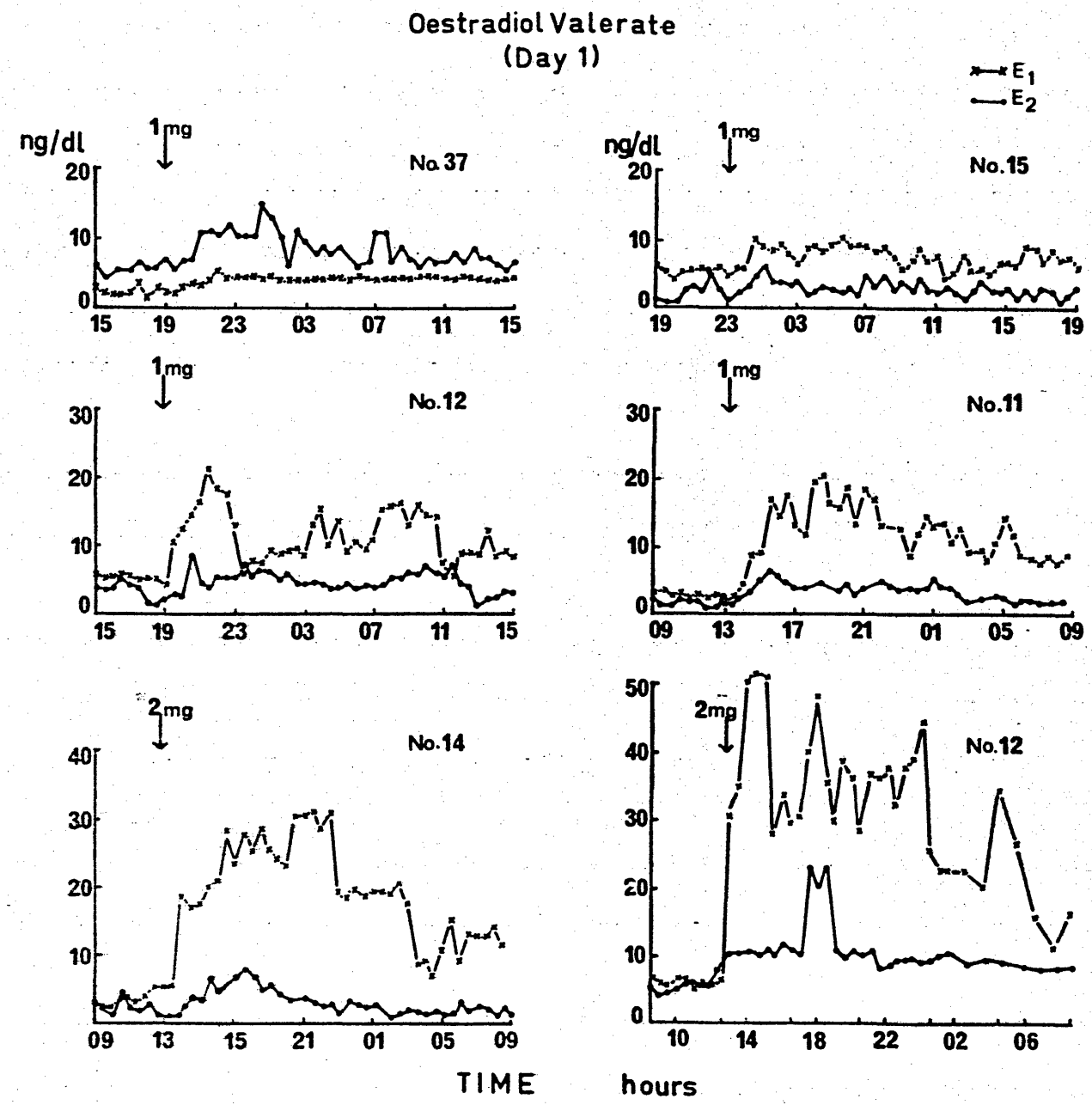

ng/dl
Piperazine Oestrone Sulphate
(Day 1)
1·5mg
No.40
No.24
No. 5
No.42
3mg
No.10
No.9
TIME hours

Figure 3.8. Plasma oestrone (×) and oestradiol (●) concentrations in six postmenopausal or ovariectomised women prescribed oestradiol valerate on the first day of a three week treatment cycle.

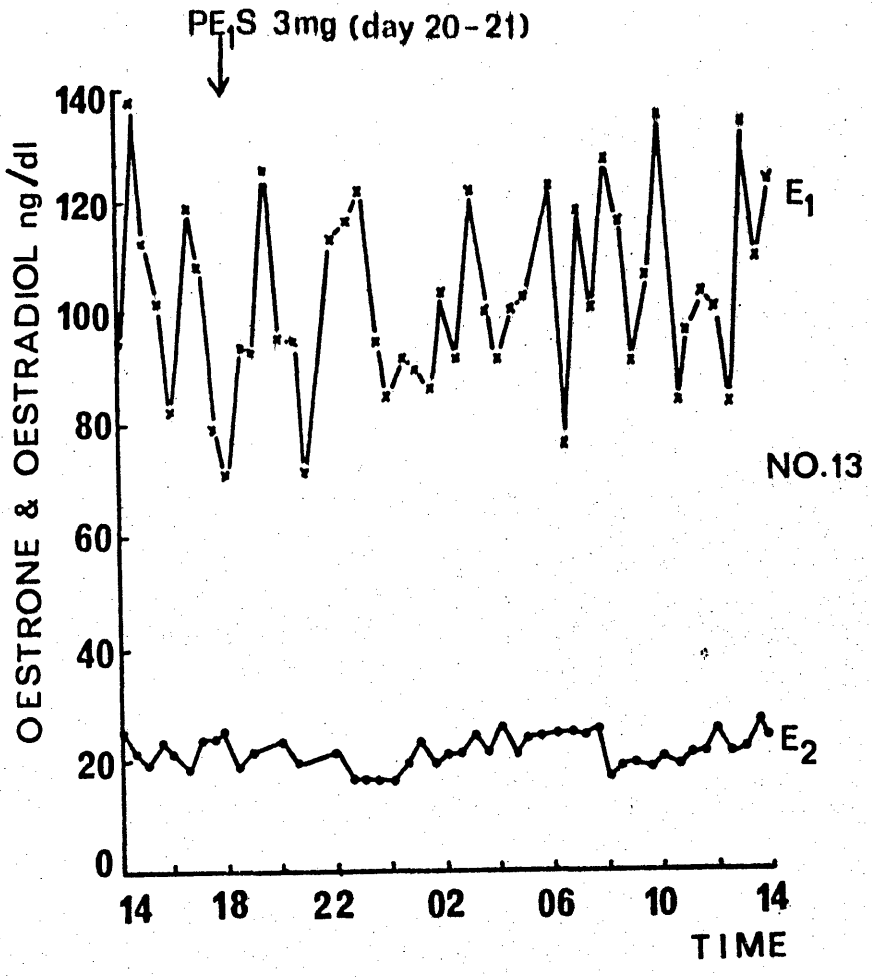

Oestradiol Valerate
(Day 1)
E1
E2
ng/dl
1mg
No. 37
No.15
No.12
No.11
2mg
No.14
No.12
TIME
hours

However, in two women given 1 mg of oestradiol valerate (Subject Nos. 15 & 37 - Figure 3.8.) plasma oestrone concentrations rose only slightly. Any increase of plasma oestradiol levels after ingestion of either of the oestrogen preparations was associated with a more marked increase of the plasma oestrone concentrations. By the completion of the sampling period, plasma oestradiol levels had usually returned to near their pretreatment levels although plasma oestrone levels were still often markedly elevated.

The effect on plasma oestrone and oestradiol concentrations of piperazine oestrone sulphate or oestradiol valerate administered towards the end of the treatment cycles are shown in Figures 3.9. and 3.10. for groups III and IV respectively. Prior to ingestion of the medication, all three women prescribed piperazine oestrone sulphate and one woman (Subject No. 38) prescribed oestradiol valerate had plasma oestrone and oestradiol concentrations that were > 35 ng/dl and >10 ng/dl respectively. After ingestion of the tablets there was only a slight increase in the plasma oestrogen levels. Two of these four women had vaginal bleeding which was associated with either cystic hyperplasia (Subject No. 13) or adenomatous hyperplasia (Subject No. 38). In two women prescribed oestradiol valerate (Subject Nos. 11 and 15), plasma oestradiol concentrations were about 6 ng/dl prior to ingestion of the dose, and the subsequent slight increase in these levels was associated with a more obvious increase in the plasma oestrone concentrations.

After ingestion of the oestrogen preparations, plasma oestrone concentrations often showed marked fluctuations,

Figure 3.9. Plasma oestrone (X) and oestradiol (●) concentrations in three postmenopausal women prescribed piperazine oestrone sulphate (PE_1S) on the 20th or 21st day of cyclical therapy.


PE1S 3mg (day 20-21)
OESTRONE & OESTRADIOL ng/dl
140
120
100
80
60
40
20
0
E1
E2
NO.13
14
18
22
02
06
10
14
TIME

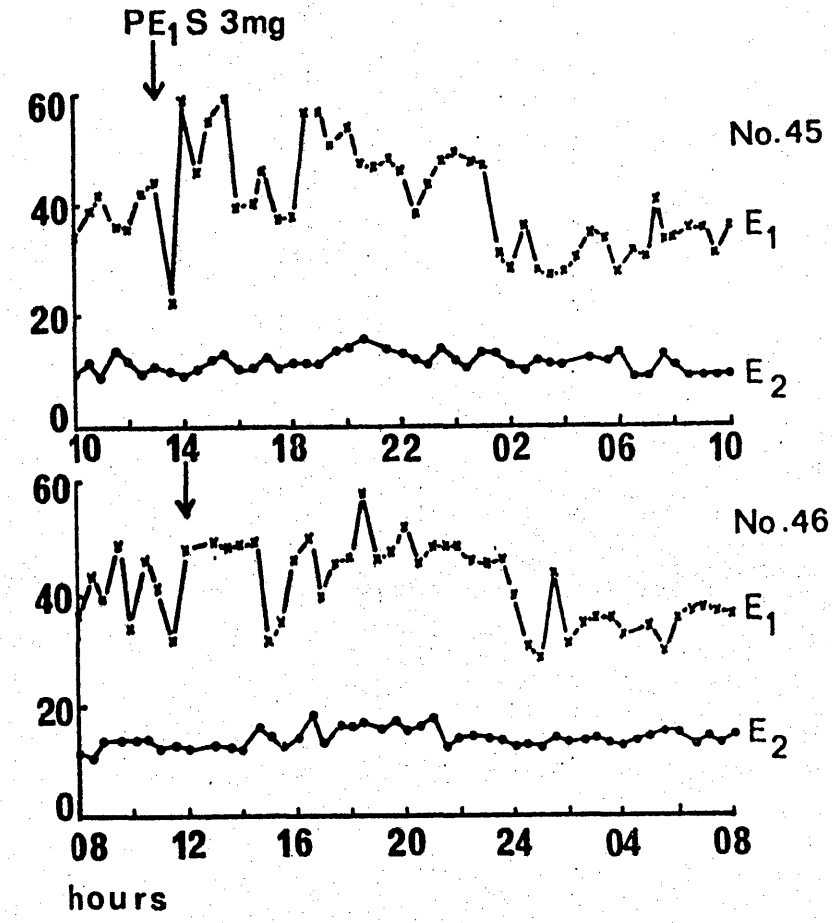
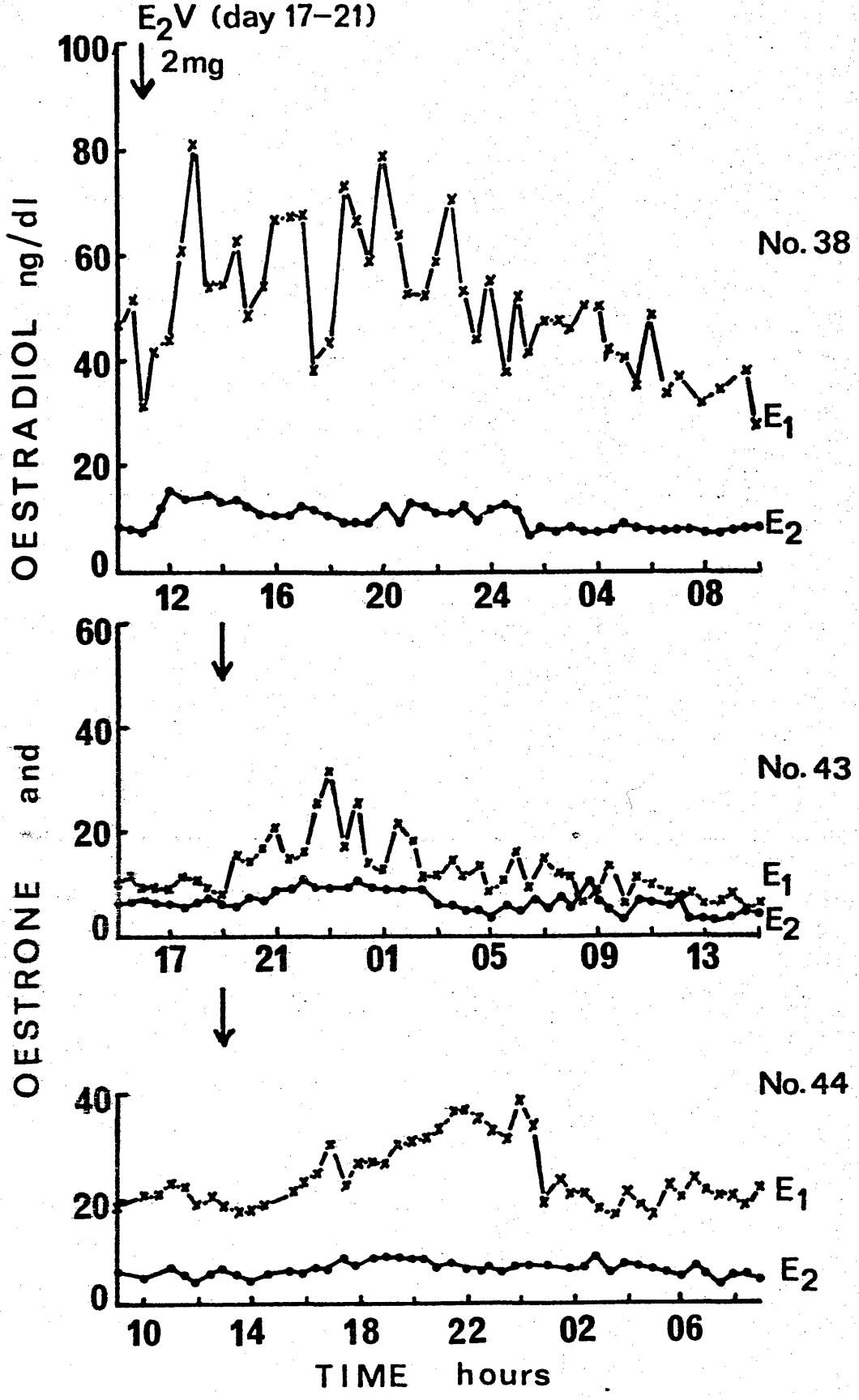

PE1S 3mg
60
40
20
0
No.45
E1
E2
10
14
18
22
02
06
10
No.46
08
12
16
20
24
04
08
hours

Figure 3.10. Plasma oestrone (✕) and oestradiol (●) concentrations in three postmenopausal or ovariectomised women prescribed oestradiol valerate (E_2V) on the 17th (No. 38), 20th (No. 44) or 21st (No. 43) day of cyclical therapy.


E2V (day 17–21)
2mg
OESTRADIOL ng/dl
OESTRONE and
No. 38
No. 43
No. 44
E1
E2
100
80
60
40
20
0
12
16
20
24
04
08
17
21
01
05
09
13
10
14
18
22
02
06
TIME hours

whereas fluctuations in the plasma oestradiol levels were, with the exception of one fluctuation in Subject No. 41 (Figure 3.8.) relatively slight.

The mean plasma oestrone and oestradiol concentrations in the four hours before, and the 20 hours after the ingestion of the oestrogen preparations in the nine women who were experiencing flushes at the time of the study are detailed in Table 3.5. The mean plasma oestrone, but not oestradiol concentrations after the ingestion of the oestrogen in the four women who experienced relief of their flushes during the ensuing cycle (i.e. Subject Nos. 9,10,14 and 42) were significantly higher ($P < 0.01$ - Mann Whitney U test) than the levels in the other five women, and flushes were relieved in the ensuing cycle of oestrogen treatment if the mean plasma oestrone levels exceeded 15 ng/dl in the 20 hours after the ingestion of the therapy on Day 1.

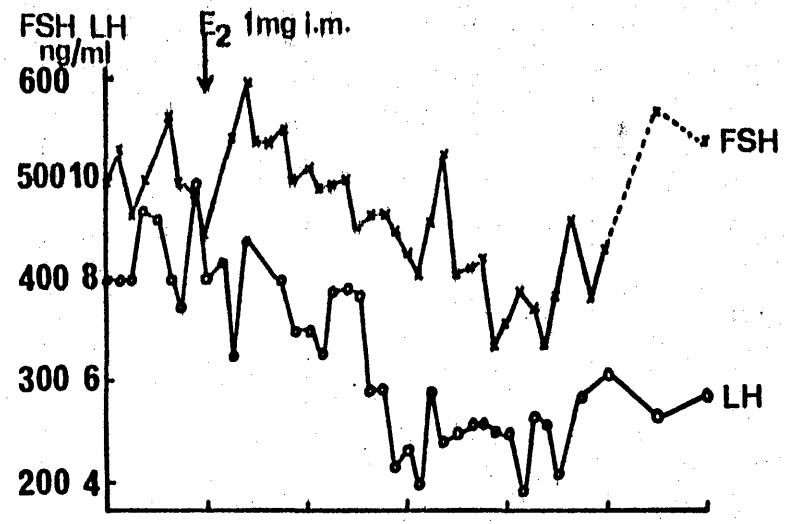
Parenteral: The plasma oestrone and oestradiol concentrations in the three women infused oestrone or oestradiol are described fully in Part 2 d and shown in Figures 2.38, 2.39 and 2.40. The mean levels before, during and after the infusions are detailed in Table 2.12. In brief, during the oestradiol infusions, plasma oestradiol concentrations rose to about 15 ng/dl whereas the plasma oestrone levels rose from 1 ng/dl to about 4 ng/dl. During the oestrone infusion, the plasma oestrone levels rose to about 14 ng/dl whereas the plasma oestradiol levels rose from 2 ng/dl to only 3 ng/dl.

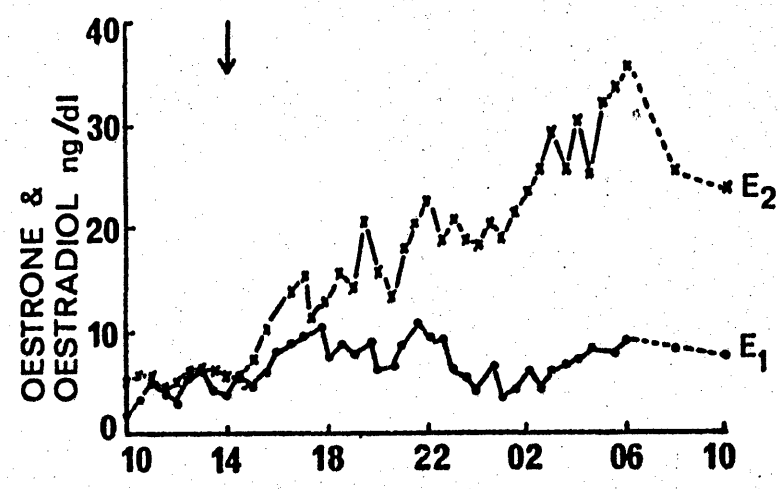
In the woman given the injection of oestradiol benzoate (1 mg), the plasma oestrone concentrations (Figure 3.11.) were in the normal postmenopausal range, but the plasma oestradiol

Table 3.5. Mean ± SD plasma oestrone and oestradiol concentrations in blood samples obtained every 30 minutes for four hours before ingestion (Pre) and in the 20 hours after ingestion (Post) of either piperazine oestrone sulphate (PE_1S) or oestradiol valerate (E_2V) from nine women who were experiencing flushes at the time of study. (R denotes relief of flushes during the subsequent treatment cycle. Differences between pre and post-ingestion levels are denoted *** $P<0.001$, ** $P<0.01$, * $P<0.05$. M = Mann Whitney U Test).

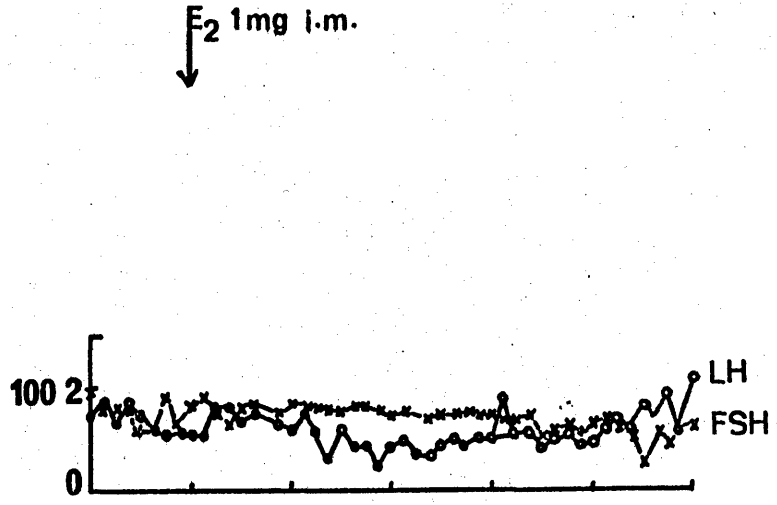
NO.	OESTROGEN PREPARATION			OESTRONE		OESTRADIOL	
	Type	Dose	Day	Pre	Post	Pre	Post
9 R	PE_1S	3.0mg	1	7.0 ± 1.6	36.2 ± 12.4***(M)	1.7 ± 1.0	4.4 ± 2.4**(M)
10 R	PE_1S	3.0mg	1	1.7 ± 0.9	19.3 ± 5.8***(M)	4.2 ± 0.8	5.8 ± 1.5**(M)
14 R	E_2V	2.0mg	1	3.7 ± 1.0	18.9 ± 7.7***(M)	2.2 ± 1.0	3.1 ± 1.9
42 R	PE_1S	1.5mg	1	12.5 ± 2.8	22.2 ± 7.7***(M)	8.6 ± 0.9	8.5 ± 1.9
5	PE_1S	1.5mg	1	3.7 ± 1.1	10.6 ± 7.0***(M)	2.4 ± 0.6	3.3 ± 1.8
11	E_2V	1.0mg	1	2.9 ± 0.5	12.2 ± 4.2***(M)	1.9 ± 0.6	3.5 ± 1.2***
12	E_2V	1.0mg	1	5.4 ± 0.4	11.6 ± 3.6***(M)	3.3 ± 1.4	4.7 ± 1.4*(M)
37	E_2V	1.0mg	1	5.7 ± 0.9	8.5 ± 2.4***(M)	2.4 ± 0.7	4.1 ± 0.5***
43	E_2V	2.0mg	21	9.9 ± 1.1	13.0 ± 6.1	7.2 ± 1.1	7.2 ± 2.2

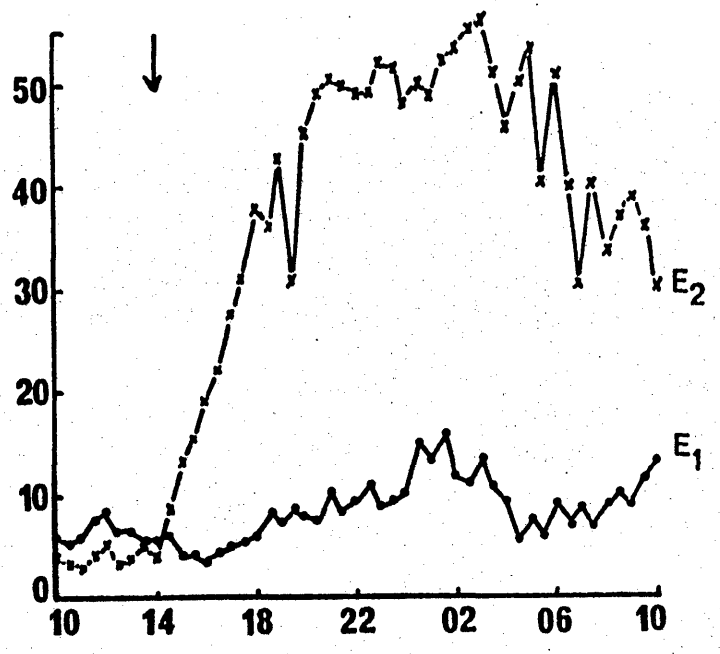
Figure 3.11. Plasma oestrone (E_1), oestradiol (E_2), FSH and LH concentrations in Subject No. 36 studied on 11.1.77 and on 5.1.77, after treatment with ethinyl oestradiol for three cycles (0.03 mg daily). (E_2 1 mg denotes an intramuscular injection of oestradiol benzoate).





(11.1.77)





(5.4.77) after 3 treatment cycles

concentrations were higher than normally found in postmenopausal women. This subject was obese (weight 71 Kg, fat mass 17 Kg), and her menopause had occurred 1.8 years previously. In the 16 hours after the injection, the plasma oestradiol concentrations rose to 36 ng/dl but the associated rise in the plasma oestrone concentrations to a mean of 6.9 ± 1.7 (SD) ng/dl was relatively slight although significant ($P < 0.001$ - Mann Whitney U test, Table 3.7.) when compared with the pre-injection levels (mean 4.3 ± 1.0 ng/dl).

The plasma oestrone and oestradiol concentrations in Subject Nos. 17 and 34 given the oestradiol implants, are shown in Figures 3.12. and 3.13. respectively and are detailed in Table 3.6. The pretreatment plasma oestrone and oestradiol concentrations were similar to those seen in ovariectomised women (Part 2 b). Within one week of the implant, plasma oestradiol concentrations rose to levels normally seen in premenopausal women during the mid-follicular phase of the ovulatory cycle and were maintained at about these levels for more than 20 weeks. During the first 12 weeks, plasma oestrone levels rose slightly and only in Subject No. 17 did oestrone become quantitatively the dominant circulating oestrogen, although the levels were still only at the upper limit of the normal range for postmenopausal women. In the four weeks after the second implant (Subject No. 17 - Figure 3.12.), the plasma oestradiol concentration rose to 13.9 ng/dl, which is comparable to late follicular phase levels in premenopausal women. The plasma oestrone levels, however, remained at about 8 ng/dl.

ii) Effects on Plasma Gonadotrophin Concentrations

Oral: The hormone profiles showing the plasma oestrogen and

Table 3.6. Mean plasma oestrone (E_1), oestradiol (E_2), FSH and LH concentrations in blood samples obtained before, and at intervals after subcutaneous implantation of oestradiol (25mg) in two ovariectomised women. After 21 weeks, a second implant of oestradiol was inserted in Subject No. 17 together with 50mg of testosterone.

	Week	E_1 ng/dl	E_2 ng/dl	FSH ng/ml	LH ng/ml
Subject No. 17					
	0	7.1	3.3	575	10.5
	1	7.4	8.8	355	8.0
	3	6.4	9.4	380	8.5
	5	7.4	7.7	325	6.5
	8	7.4	5.8	290	5.8
	10	7.4	6.2	325	7.0
	12	8.7	6.1	300	7.3
	14	8.8	4.7	195	9.0
	17	7.7	6.3	260	5.8
	21	7.5	6.8	265	6.3
	(23)	8.7	11.4	250	8.0
	(25)	8.2	13.9	145	4.5
Subject No. 34					
	0	2.9	2.4	370	5.1
	1	2.9	5.7	140	6.4
	3	3.5	6.2	31	0.5
	7	4.9	5.6	23	1.4
	13	6.7	6.8	80	1.0
	30	4.0	4.9	94	1.4

Figure 3.12. Plasma oestrogen and gonadotrophin concentrations in Subject No. 17 after subcutaneous implantation of oestradiol (25 mg) on two occasions, the second of which was combined with testosterone (50 mg).

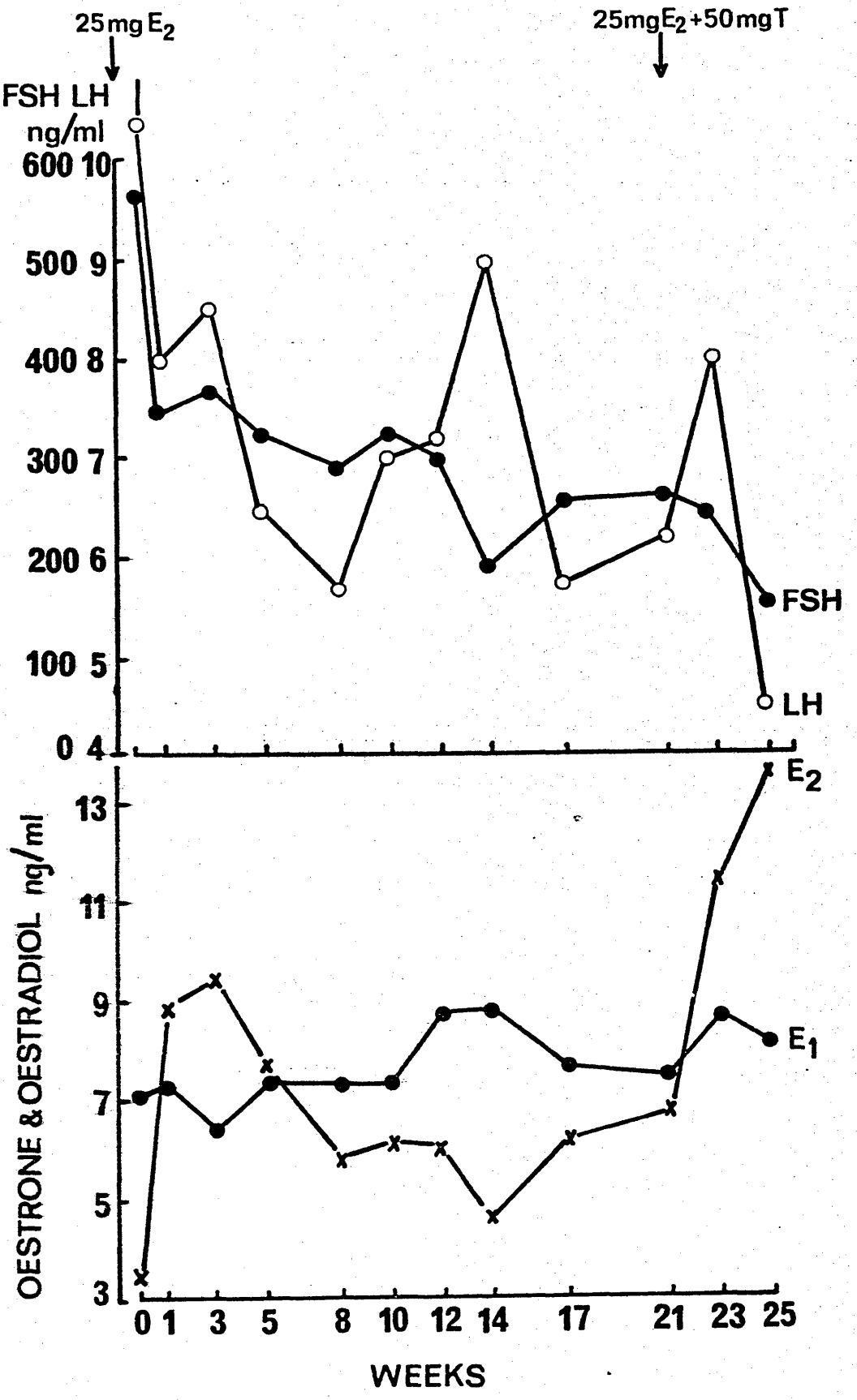

25mg E2
25mgE2+50mgT
FSH LH
ng/ml
600 10
500 9
400 8
300 7
200 6
100 5
0 4
FSH
LH
E2
E1
OESTRONE & OESTRADIOL ng/ml
13
11
9
7
5
3
0 1 3 5 8 10 12 14 17 21 23 25
WEEKS

Figure 3.13. Plasma oestrogen and gonadotrophin concentrations in Subject No. 34 after subcutaneous implantation of oestradiol (25 mg).

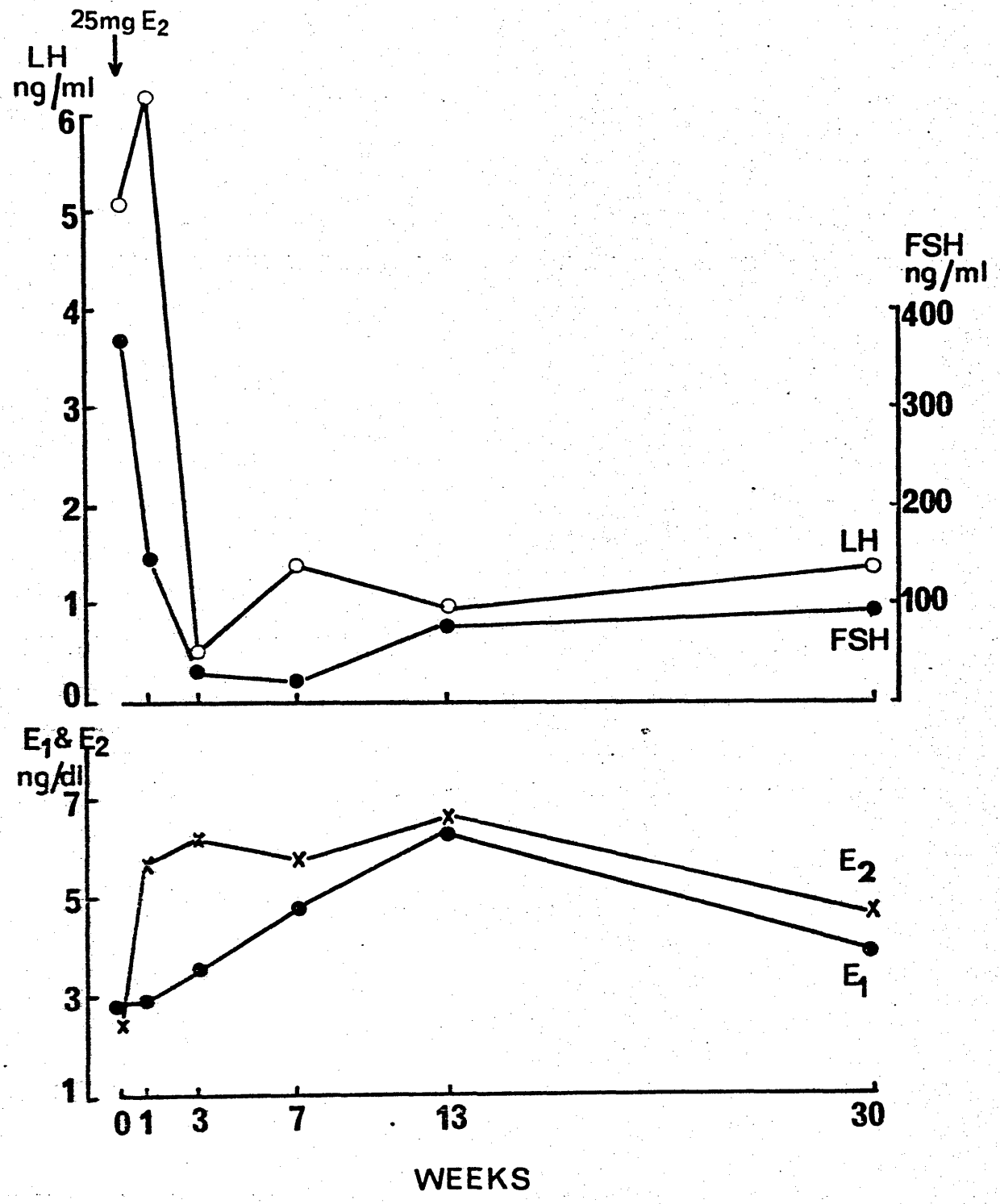

25mg E_2
LH
ng/ml
6
5
4
3
2
1
0
FSH
ng/ml
400
300
200
100
LH
FSH
E_1 & E_2
ng/dl
7
5
3
1
E_2
E_1
0 1 3 7 13 30
WEEKS

Table 3.7. Mean ± SD plasma oestrone (E_1), oestradiol (E_2), LH and FSH concentrations in blood samples obtained every 30 minutes for four hours before ingestion (Pre) and in the 16 hours after ingestion (Post) of oestradiol benzoate 1mg in Subject No. 36 who was studied on two occasions, 11.1.77 and then 5.4.77 after 11 weeks of cyclical treatment with ethinyl oestradiol 0.03mg daily, the last dose having been taken twelve hours before the commencement of the sampling period. (Differences between pre- and post-ingestion levels are denoted *** $P<0.001$, ** $P<0.01$, * $P<0.05$. (M) = Mann Whitney U test).

DATE	SAMPLING PERIOD	E_1 ng/dl	E_2 ng/dl	LH ng/ml	FSH ng/ml
11.1.77	Pre	4.3 ± 1.0	5.5 ± 0.6	8.5 ± 0.9	499 ± 33
	Post	6.9 ± 1.7***(M)	20.5 ± 7.0***(M)	5.9 ± 1.6***(M)	456 ± 68*(M)
5.4.77	Pre	6.8 ± 0.9	4.4 ± 0.7	1.5 ± 0.2	78 ± 11
	Post	8.6 ± 3.2*(M)	42.4 ± 13.7***(M)	1.0 ± 0.4**(M)	69 ± 10

gonadotrophin concentrations of the four women (Subject Nos. 5,9, 14 & 24) are shown in Figures 3.14., 3.15., 3.16. & 3.17. respectively. The plasma oestrone and oestradiol concentrations in these four subjects have been described above. The FSH and LH concentrations in all subjects were within the normal postmenopausal range throughout the sampling period and showed the usual fluctuations suggestive of episodic production, as discussed in Part 2 a. However, many of the fluctuations in plasma FSH and LH concentrations in Subject Nos. 9 & 24 (Figures 3.15 & 3.17 respectively) appeared synchronous, but this was not apparent in the other subjects. However, Subject No. 5 (Figure 3.14.) had many major fluctuations in LH and oestradiol concentrations that appeared synchronous, but this was not apparent in the other three subjects. The mean levels of oestrone, oestradiol, FSH and LH before and after ingestion of the oestrogen preparations are detailed in Table 3.8. The only significant difference of the mean gonadotrophin concentrations before and after ingestion of the oestrogens was in Subject No. 9 in whom the fall in FSH levels was associated with the only significant increase in the plasma oestradiol concentrations ($P < 0.01$).

Parenteral: The plasma FSH and LH concentrations in the two women (Subject Nos. 32 & 33) infused oestradiol are shown in Figures 2.38. and 2.39. respectively, and in Subject No. 34 who was infused with oestrone in Figure 2.40. The mean oestrone, oestradiol, FSH and LH concentrations before the infusion, during the last four hours and in a four hour period after the infusion are detailed in Table 2.12. The changes in the oestrone and oestradiol levels have been described previously. The plasma

Figure 3.14. Plasma oestrone (●), oestradiol (✕), FSH and LH concentrations in Subject No. 5 prescribed piperazine oestrone sulphate (PE_1S) on Day 1.

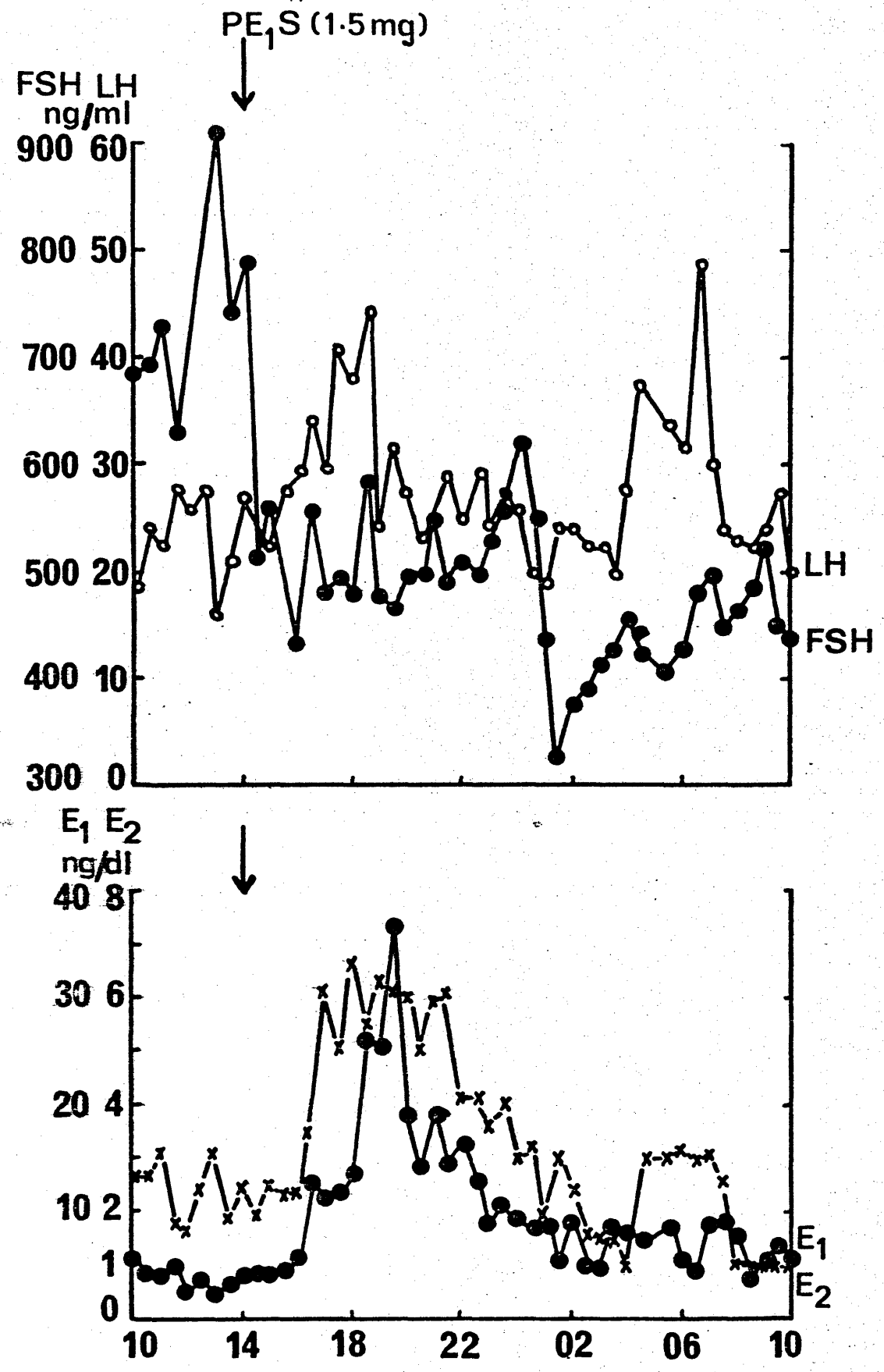

PE1S (1·5 mg)
FSH LH
ng/ml
900 60
800 50
700 40
600 30
500 20
400 10
300 0
LH
FSH
E1 E2
ng/dl
40 8
30 6
20 4
10 2
1
0
E1
E2
10
14
18
22
02
06
10

Figure 3.15. Plasma oestrogen and gonadotrophin concentrations in Subject No. 9 administered piperazine oestrone sulphate (PE_1S).

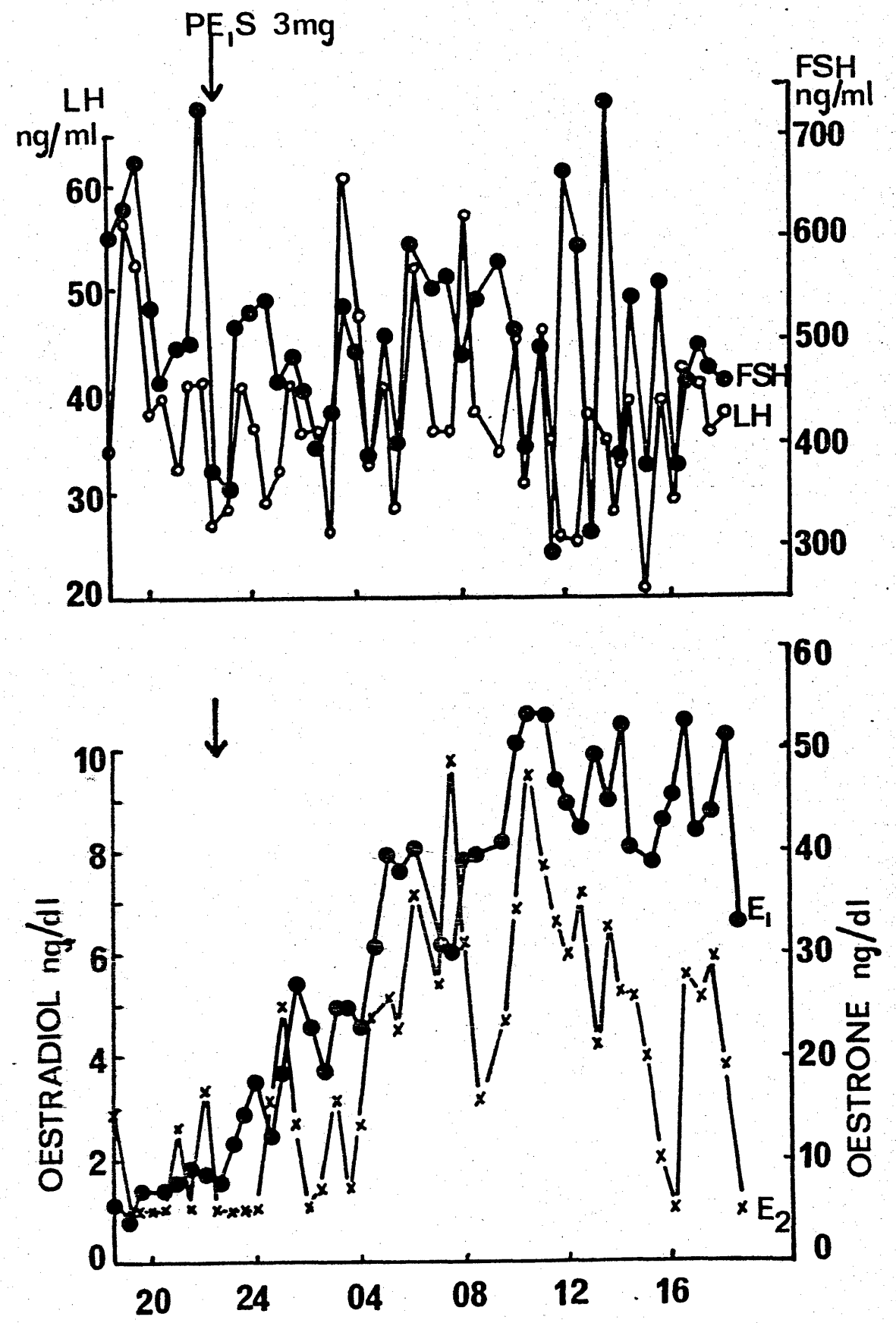

PE1S 3mg
LH
ng/ml
FSH
ng/ml
60
50
40
30
20
700
600
500
400
300
FSH
LH
OESTRADIOL ng/dl
OESTRONE ng/dl
10
8
6
4
2
0
60
50
40
30
20
10
0
E1
E2
20
24
04
08
12
16

Figure 3.16. Plasma oestrogen and gonadotrophin concentrations in Subject No. 14 prescribed oestradiol valerate (E_2V).

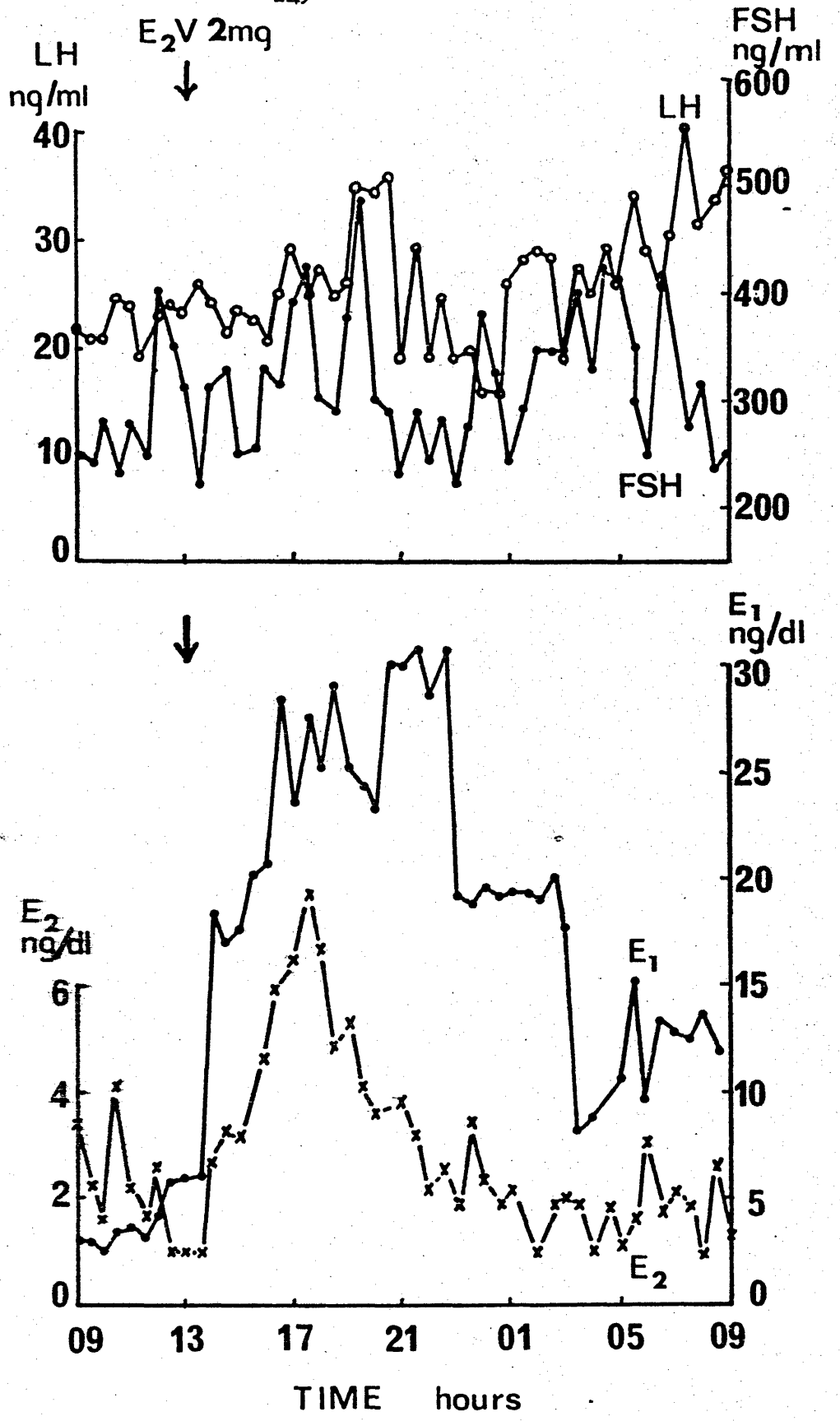

LH
ng/ml
E_2V 2mg
FSH
ng/ml
LH
FSH
40
30
20
10
0
600
500
400
300
200
E_1
ng/dl
E_2
ng/dl
30
25
20
15
10
5
0
6
4
2
0
E_1
E_2
09
13
17
21
01
05
09
TIME hours

Figure 3.17. Plasma oestrogen and gonadotrophin concentrations in Subject No. 24 prescribed piperazine oestrone sulphate (PE_1S).

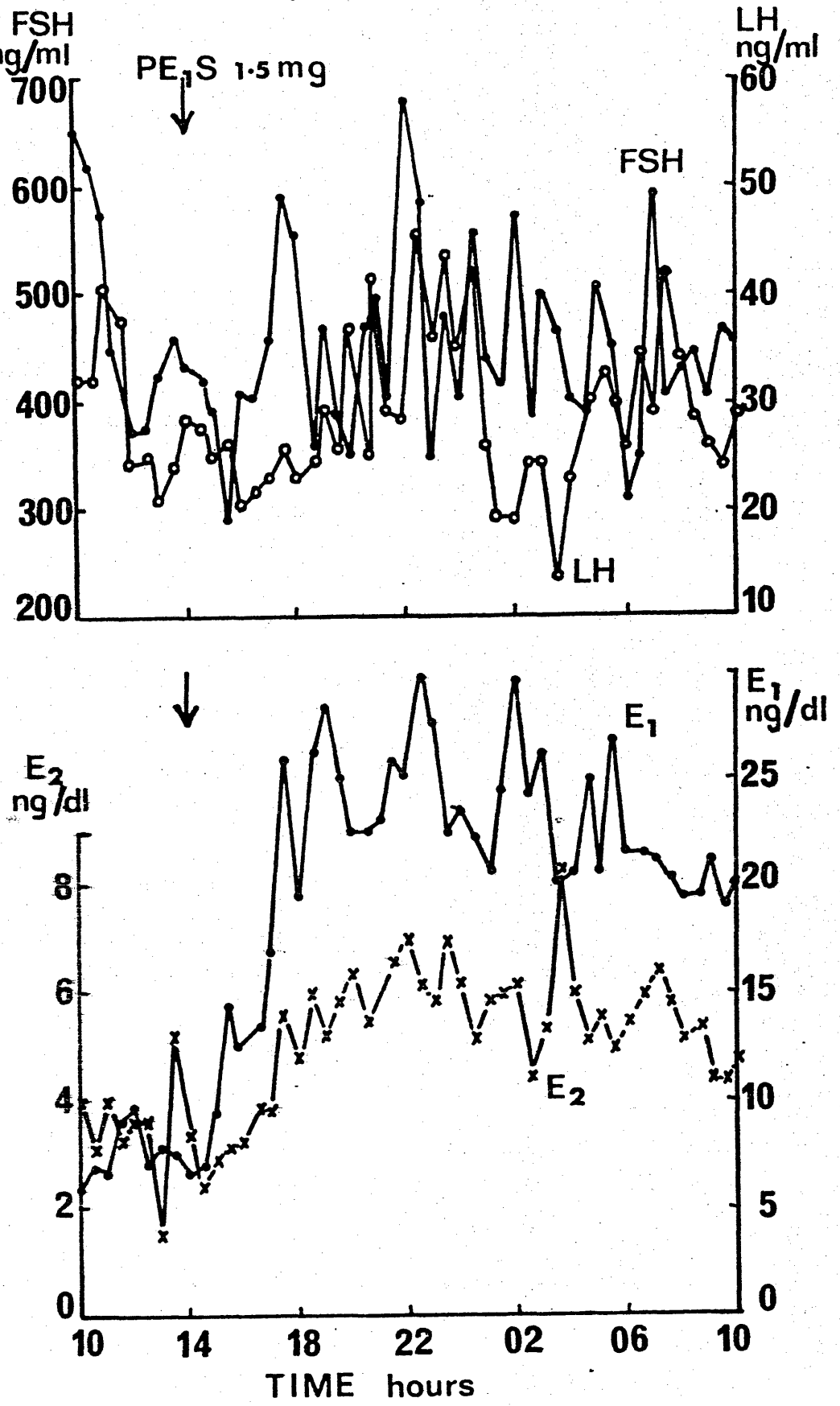

FSH
ng/ml
PE1S 1·5 mg
LH
ng/ml
700
600
500
400
300
200
60
50
40
30
20
10
FSH
LH
E2
ng/dl
E1
ng/dl
8
6
4
2
0
25
20
15
10
5
0
E1
E2
10
14
18
22
02
06
10
TIME hours

Table 3.8. Mean ± SD plasma oestrone (E_1), oestradiol (E_2), LH and FSH concentrations in blood samples obtained every 30 minutes for four hours before ingestion (Pre) and in the 20 hours after ingestion (Post) of piperazine oestrone sulphate (1.5mg Subject Nos. 5 & 24, 3mg Subject No. 9) or oestradiol valerate (2mg Subject No. 14). Differences between pre- and post-ingestion levels are denoted *** $P<0.001$, ** $P<0.01$ - (M) = Mann Whitney U test.

NO	SAMPLING PERIOD	E_1 ng/dl	E_2 ng/dl	LH ng/ml	FSH ng/ml
5	Pre	3.7 ± 1.1	2.4 ± 0.6	40.7 ± 9.7	554 ± 113
	Post	10.6 ± 7.0***(M)	3.3 ± 1.8	37.5 ± 8.9	484 ± 96
9	Pre	7.0 ± 1.6	1.7 ± 1.0	23.5 ± 4.0	749 ± 83
	Post	36.2 ± 12.6***(M)	4.4 ± 2.4**(M)	27.1 ± 6.0	478 ± 62***
14	Pre	3.7 ± 1.0	2.2 ± 1.9	22.3 ± 2.2	289 ± 57
	Post	18.9 ± 7.7***(M)	3.1 ± 1.9	26.7 ± 5.7	320 ± 65
24	Pre	7.4 ± 1.1	3.6 ± 1.0	29.6 ± 6.7	484 ± 104
	Post	21.2 ± 6.1***(M)	5.4 ± 1.2	29.1 ± 7.4	442 ± 77

FSH and LH concentrations before the infusion were similar to those seen in normal postmenopausal women. However in the two women infused oestradiol, LH, but not FSH, levels not only fell significantly ($P < 0.05$) towards the end of the infusion, but also remained significantly lower ($P < 0.01$) after the completion of the infusion when compared to pre-infusion levels. In one subject (No. 33) plasma FSH concentrations were also significantly lower ($P < 0.01$) than the pre-infusion value. Similar changes did not occur in the woman infused oestrone.

The plasma hormone profile of the woman (Subject No. 36) injected with oestradiol benzoate (1 mg) is shown in Figure 3.11. Plasma LH concentrations showed a more significant fall ($P < 0.001$) than the plasma FSH ($P < 0.05$) in the sixteen hours after the injection (Table 3.7.) when compared to pre-infusion levels. The treatment with ethinyl oestradiol for three cycles resulted in a fall in the plasma FSH and LH concentrations to levels normally seen in premenopausal women. The mean plasma oestradiol concentrations were significantly lower ($P < 0.01$) and the mean oestrone concentrations were significantly higher ($P < 0.001$) in the pre-injection period after treatment with ethinyl oestradiol when compared with the pre-injection levels before any treatment. The treatment with ethinyl oestradiol also resulted in a fall in the plasma FSH and LH concentrations to levels normally seen in premenopausal women. The injection of oestradiol on 5.4.77 was associated with further significant falls in the plasma LH levels ($P < 0.01$) and the FSH level ($P < 0.05$) when compared with the levels in the four hours preceding the injection. The plasma oestradiol and oestrone concentrations after the second

injection were, however, significantly higher ($P < 0.001$) than those found after the first injection.

The plasma concentrations of the gonadotrophins in the subjects treated with the oestradiol implants are shown in Figures 3.12. and 3.13. and detailed in Table 3.6. Plasma FSH and LH concentrations in Subject No. 34 (Figure 3.13.) showed a prompt fall to levels seen normally in premenopausal women. In the other subject (Figure 3.12.) the plasma FSH and LH concentrations fell from their pre-treatment values although, even within four weeks of the second implant, the concentrations were not quite within the range for premenopausal women.

DISCUSSION

i) Effect on Plasma Oestrogen Concentrations

The results of this study confirm the findings of Anderson et al. (1978) that oral treatment with piperazine oestrone sulphate and oestradiol valerate produces similar changes in the plasma concentrations of the unconjugated oestrogens. Thus the oral administration of both these oestrogen preparations markedly increased the plasma oestrone concentrations and any increase in the plasma oestradiol concentrations was comparatively modest. This preferential increase of the plasma oestrone concentrations after the ingestion of oestradiol valerate has also been observed by Englund and Johansson (1977) and Spona and Schneider (1977), is similar to that produced by micronised oestradiol (Yen et al., 1975) and may result from oxidation of oestradiol to oestrone in the gut, especially as *in vitro* studies have shown that ileal tissue can oxidise oestradiol to oestrone (Ryan and Engel, 1953).

The results of this study suggest that some of the oestrone absorbed into the circulation may then be converted in peripheral tissues to oestradiol, although the study by Anderson et al. (1978) indicated that much of this absorbed oestrone was rapidly conjugated because the increase in the plasma concentration of oestrone sulphate was much greater than that of oestrone. It is of interest, however, that Kicovic et al. (1977) found that the oral administration of oestradiol decanoate did not produce the unphysiological plasma oestrone: oestradiol ratios that occurred after the oral administration of piperazine oestrone sulphate, oestradiol valerate or micronised oestradiol (as discussed above) . This may be because, like testosterone decanoate (Coert et al., 1975),this oestradiol ester is incorporated into the cyclomicrons of the intestinal wall and, thus protected from oxidation, is then transported in the lymph to the peripheral circulation.

Considerable variations in the plasma oestrone and oestradiol concentrations occurred in the women in this study. Similar variations have been previously observed in women prescribed oestradiol valerate (Englund & Johansson, 1977), and, plasma oestradiol concentrations in blood samples obtained at the same stage of the treatment in women prescribed piperazine oestrone sulphate also vary considerably (Cooper et al., 1974). These individual variations, which may be due to differences in the absorption or clearance of these oestrogens, suggests that different doses of the oestrogen preparations may be required by different women to relieve climacteric symptoms.

In this study flushes were relieved during the treatment

cycle when the mean plasma oestrone concentrations exceeded 15 ng/dl in the 20 hours after the ingestion of the first dose However, it must be accepted that the plasma oestrone and oestradiol concentrations in women studied on the first day of the treatment cycle were usually quite different from those observed later in the treatment cycle. Also studies described in Part 3 a suggested that plasma oestrone did not have a direct role in the pathogenesis of flushes. However, the findings in the present study would be consistent with the hypothesis that flushes may be related to an interaction of oestrogen metabolites with sympathetic amines in the central nervous system, especially as in vitro studies have shown that the intracerebral concentrations of catechol oestrogens in animals may be much higher than their parent compounds (Paul and Axelrod, 1977).

Although oestrone has been suggested as being a specific endometrial carcinogen (Siiteri et al., 1974; Ziel and Finkle, 1976), endometrial carcinoma has been described in association with synthetic oestrogen (Wilkinson et al., 1973; Reid and Shirley, 1974) and with abnormal production of oestradiol (but not of oestrone) by a thecoma (Reed et al., 1978). Furthermore, oestrone is biologically a less potent oestrogen than oestradiol (Fotherby, 1976). Therefore the development of endometrial hyperplasia, as occurred in two women in this study prescribed oral oestrogen therapy, may have been related more to the high plasma levels of oestradiol, whose endometrial proliferative effects were not antagonised by cyclical progestogen, than to the high plasma oestrone concentrations per se. The finding of high

plasma oestrogen levels in the women prescribed the higher doses of oestrogen is, however, consistent with the findings in recent studies of a direct relationship of the dose of therapy and the incidence of endometrial neoplasia (Ziel and Finkle, 1975; Gray et al., 1977). However, further studies are necessary to determine whether monitoring the plasma oestrogen concentrations so that there is repletion of the circulating deficiency of oestrogens by the therapy, whilst alleviating the symptoms and signs of primary ovarian failure, can be achieved without inducing side effects such as endometrial hyperplasia.

In this study, the treatment with piperazine oestrone sulphate or oestradiol valerate in the usually recommended doses did not fulfil either the qualitative or quantitative concepts implicit in the term 'hormone replacement therapy', as the plasma oestradiol levels in women prescribed such therapy often exceeded the levels of about 3.8 ng/dl observed in the asymptomatic postmenopausal women studied in Part 3 a, and were occasionally higher than the levels of 8-10 ng/dl seen during the follicular phase of the ovulatory cycle. Furthermore, the plasma oestrone concentrations were nearly always higher than seen at other times of life (Hawkins and Oakey, 1974). However, in two women (Subject Nos. 40 & 42- Figure 3.7.) before the therapy was administered, the plasma oestradiol levels were higher than normally seen in postmenopausal women presumably because secretion by the ovary which had not become exhausted of follicles despite raised gonadotrophin levels that were suggestive of primary ovarian failure.

This study also showed that the parenteral administration of oestradiol resulted in a different plasma oestradiol: oestrone

ratio than occurred after the oral administration of oestradiol, thus contradicting the conclusion of iso topic excretion studies by Fishman et al. (1969) that oral oestradiol was probably metabolised like intravenously administered oestradiol. The ratio of oestradiol:oestrone of about 4:1 after the intravenous or intramuscular administration of oestradiol is consistent with the conversion ratio of oestradiol to oestrone found by Longcope et al. (1968) in premenopausal women. The plasma oestradiol: oestrone ratio of about 1:1 after implantation of the oestradiol pellets was different from the ratio after the intravenous administration. Rigg et al. (1977 & 1978) found that the ratio after the intranasal administration of oestradiol was also about unity whereas the ratio after the intravaginal administration was similar to that reported after the infusion or injections of oestradiol. The finding in the present study of oestradiol: oestrone ratios of about 1:5 after either oral or intravenous administration of oestrone was also observed by Ruder et al. (1972) after the oral or intravenous administration of isotopically labelled oestrone sulphate. However differences in the absorption or metabolism of oestrogen administered by different routes may become important only if future studies show that plasma oestrone has a major biological role. Meanwhile, most of the biological effects of the administration of 'natural' oestrogens by different routes probably depends primarily on the plasma oestradiol concentration attained with the therapy.

The longitudinal studies of the women given 25 mg oestradiol implants (Figures 3.12. & 3.13.) showed that the effect on the plasma oestrogen concentrations may sometimes be for a longer

period of time than the normally stated duration of effect of three to six months. Thus, to avoid high plasma oestradiol levels that might result from premature reimplantation, the plasma oestradiol levels, or alternatively the karyopyknotic index, should be determined first to ensure that nearly all the oestradiol has been released from the previous implant.

The effect of exogenous oestrogen on endogenous oestrogen production and metabolism was only studied in one subject. Nevertheless, the finding of lower plasma oestradiol concentrations in the woman after three treatment cycles of ethinyl oestradiol when compared with pretreatment levels would be consistent with the findings by Longcope et al. (1974) of an increased metabolic clearance rate of oestradiol in younger women prescribed the contraceptive pill containing 50 ug of ethinyl oestradiol. Longcope et al. (1974) attributed the increased metabolic clearance rate of oestradiol to increased rate of enzymic catabolism of oestradiol induced by ethinyl oestradiol and not to any effect of ethinyl oestradiol on binding globulins. In the study by Longcope et al.(1974), the metabolic clearance rate of oestrone was not decreased by ethinyl oestradiol (although it was by mestranol) and thus, the significance of the slightly higher oestrone concentrations that occurred after the ethinyl oestradiol treatment in the present study is unknown. The higher plasma oestrogen levels after the administration of oestradiol benzoate probably resulted from variations in the absorption rate of the oestradiol from the site of injection.

The finding in this study of a more significant fall of plasma LH than FSH concentrations after oestradiol was administered

intravenously (Figures 3.38. & 3.39.) or intramuscularly (Figure 3.11.) agrees with the findings of similar studies by Nillius and Wide (1970) and Tsai and Yen (1971), and may occur because the rate of clearance of LH is greater than that of FSH (Schalch et al., 1968 ;Coble et al., 1969). However, a significant fall in FSH may occur if pharmacological rather than physiological doses of oestradiol are infused (Tsai and Yen, 1971). A lack of the hypothetical 'FSH-inhibiting' substance ('inhibin') may explain the observation in this study and in previous studies of perimenopausal women by Sherman & Korenman (1975) and Van Look et al. (1977) of raised FSH concentrations despite raised plasma oestradiol concentrations.

Individual differences in the sensitivity of the hypothalamus and pituitary to oestrogen feedback may explain the different degrees of fall in the plasma gonadotrophin concentrations after the oestradiol implants when the plasma oestradiol concentrations in the two ovariectomised women were similar. Plasma FSH concentrations fell during the oestrone infusion (Figure 2.40.) and LH concentrations rose after the infusion, but it is unlikely that oestrone affects gonadotrophin secretion because similar changes did not occur in all the women prescribed piperazine oestrone sulphate or oestradiol valerate. The hormone profiles of the women prescribed oral oestrogen (Figures 3.14. - 3.17.) did not all show a significant fall of FSH or, more particularly, of LH after the oral administration of oestrogen preparations. This was contrary to the findings of Yen et al. (1975) who prescribed 2 mg of micronised oestradiol, that is a slightly higher dosage than that prescribed in the present study.

Nevertheless, the changes in the gonadotrophin concentrations in the women in the present study provide further support for the concept that plasma oestradiol is biologically the important plasma oestrogen in postmenopausal women, and the need for further studies to determine the biological role, if any, of plasma oestrone after the menopause.

PART 4

SUMMARY AND CONCLUSIONS

The development of the radioimmunoassay technique has allowed hormone concentrations to be easily and accurately determined in minute amounts of blood. In the radioimmunoassay methods employed in this investigation, plasma oestradiol and oestrone concentrations as low as 1-2 ng/dl could be reliably determined. Furthermore, plasma cortisol, androstenedione, testosterone, FSH and LH concentrations could also be measured by radioimmunoassay in each 7 ml blood sample. Thus profile techniques in which frequent blood samples were obtained throughout the day could be employed to study physiological and clinical aspects of plasma oestrogens after the menopause. The ultimate objective of this study was to develop a firmer biochemical basis for the practical management of postmenopausal women, particularly those experiencing climacteric symptoms.

This study confirmed the conclusions of other investigators that the major circulating hormone deficiency resulting from primary ovarian failure was of oestradiol, the mean level of which was markedly below that observed in premenopausal women during the mid-follicular phase of the ovulatory cycle. However, the mean oestradiol concentration of 3.2 ± 0.3 (SEM) ng/dl in the postmenopausal women in this study may have been higher than reported by many other investigators because in most of the women, less than five years had elapsed since their menopause. Thus the ovary may not have become completely exhausted of follicles in all

the women in this study. After hypothalamic-pituitary adrenal suppression (by dexamethasone treatment for one week), the plasma oestradiol concentration in the women within two years of their menopause was $>$ 2 ng/dl, whereas the level was $<$ 2 ng/dl in an older postmenopausal woman, and in all ovariectomised women. Also in the subject studied longitudinally after four months of amenorrhoea, follicular secretion of oestradiol occurred several months after FSH and LH concentrations had been raised into the ranges that are diagnostic of primary ovarian failure. However, as the number of women in these studies was small, ovarian secretion of oestradiol in women during the first few years after the menopause requires further study.

This study confirmed the findings in most other studies that oestrone, and not oestradiol, was quantitatively the dominant unconjugated oestrogen circulating in postmenopausal women, and that the deficiency of circulating oestrone that resulted from primary ovarian failure was small compared with the deficiency of oestradiol that occurred after the menopause. Thus, accurate fulfilment of the concept of 'hormone replacement therapy' of repairing the major hormonal deficiencies resulting from ovarian failure requires the oestrogen therapy to increase the plasma oestradiol concentration much more than the plasma oestrone concentration.

In the postmenopausal and ovariectomised women in this study, the plasma androgen and oestrogen concentrations were similar and, with the exception of the plasma oestrogens in the younger postmenopausal women, were almost unmeasurable after dexamethasone treatment for one week. This study thus

showed that the postmenopausal ovary was an insignificant source of plasma androgens and oestrogens when compared with the adrenal contribution and there is therefore no endocrine indication for the conservation of the postmenopausal ovary, at least in women in whom more than two years have elapsed since their menopause. However, further investigations are required to explain the discrepancy of these results and the findings by some other investigators (Judd et al., 1974 & 1976; Vermeulen, 1976) who concluded that there was a significant ovarian contribution to plasma androstenedione and testosterone production in postmenopausal women.

The plasma concentrations of only cortisol and androstenedione showed synchronous fluctuations in the hormone profile studies of the normal women, and these steroids were also suppressed by a single dose of dexamethasone treatment. Thus the episodic adrenal secretion of only these two steroids was directly regulated by ACTH in the postmenopausal and ovariectomised women studied. There was, however, occasional asynchrony of the fluctuations in the plasma levels of cortisol and androstenedione in the hormone profiles of normal women before and after ACTH stimulation that were probably due to differences in their clearance rates, especially as marked fluctuations in the plasma concentrations of androstenedione, but not of cortisol, occurred during a constant infusion of androstenedione and cortisol in an ovariectomised ACTH-suppressed woman.

This study also showed that there was no physiological basis for the prescription of testosterone as 'hormone replacement therapy' as there was no evidence of a circulating testosterone deficiency

resulting from ovarian failure. The plasma concentrations of testosterone in the intensive hormone profile studies showed only slight fluctuations which were not synchronous with the fluctuations in the androstenedione concentrations. Plasma testosterone levels were also not suppressed by a single dose of dexamethasone treatment. This suggests that there was no significant adrenal secretion of testosterone. However, there was a significant correlation of the plasma androstenedione and testosterone concentrations ($P < 0.05$). In addition, plasma testosterone concentrations became unmeasurable after prolonged treatment with dexamethasone and, furthermore, increased acutely during the infusions of ACTH or androstenedione. It was concluded from these observations that plasma testosterone in postmenopausal and ovariectomised women is predominantly produced from plasma androstenedione. However, further studies are required to determine the factors regulating this peripheral production.

In the profile studies, the plasma oestrone and oestradiol concentrations showed asynchronous fluctuations. Furthermore these fluctuations of oestrone and oestradiol were not synchronous with any fluctuations in the plasma levels of their androgenic prehormones, androstenedione and testosterone. Although the plasma levels of oestrone and oestradiol were not suppressed by a single dose of dexamethasone treatment, the plasma oestrogen levels became almost unmeasurable after treatment with dexamethasone for one week. The infusion of ACTH or androstenedione increased the plasma levels of oestrone and sometimes also of oestradiol. Thus these studies confirmed the conclusions of Grodin et al. (1973) that the production of plasma oestrogens, particularly oestrone,

is predominantly by the peripheral aromatisation of androstenedione. However, and contrary to the findings from isotopic studies by Longcope et al. (1969) and Olivo et al. (1973), infused testosterone was predominantly aromatised to oestrone and not to oestradiol.

The finding of a positive correlation of the plasma oestrone and oestradiol concentrations and the percentage difference from ideal body weight (reflecting fat mass) confirmed the conclusion of some other studies (Siiteri and MacDonald, 1973; MacDonald et al., 1978) that the amount of adipose tissue affected the production of plasma oestrogens after the menopause. Further studies are, however, necessary to determine more exactly the factors regulating the peripheral production in, and release from, the aromatising tissues. Further studies are also required to determine the role of thyroid hormones in the peripheral production of plasma oestrogens after the menopause. This is particularly so because of the finding in a hyperthyroid woman of increased plasma oestradiol, but not oestrone, levels. This is in contrast to the results of isotopic studies by Southren et al., 1974 that have shown that hyperthyroidism is associated with an increased conversion of androgens to oestrone, but not to oestradiol.

It is uncertain from the results of this study whether the fluctuations observed in the plasma oestrogen levels resulted from fluctuations in the clearance of these unconjugated plasma oestrogens, or from episodic release of the oestrogens from the aromatising tissues. After the infusion of androgens, or of ACTH, plasma levels of oestradiol, and more particularly of oestrone, often remained elevated long after the infusions whereas this did not occur after infusions of oestradiol or oestrone. Thus, release

of plasma oestrogens from the aromatising tissues was prolonged. These findings also suggest that if infused oestrogens are conjugated or taken up in adipose tissue in significant amounts (as shown in isotopic studies by Vaughan et al. (1976) and Twombley and Levitz (1967) respectively) neither of these are major sources of unconjugated plasma oestrogens in postmenopausal women. However, the physiological role of plasma conjugates was not examined directly in the present study and therefore further studies are necessary to determine the exact biological significance of the interconversion of oestrone sulphate with plasma oestrone and oestradiol.

In the present study, the fluctuations of the plasma FSH and LH levels and gonadotrophin and steroid levels were asynchronous. Whilst these findings confirmed the observations of other investigators that plasma gonadotrophin secretion was episodic, they also demonstrated the need for further studies of the factors regulating the episodic secretion of gonadotrophins, and the possible role of plasma gonadotrophins in postmenopausal women.

The clinical studies of the relationship between plasma oestrone and oestradiol, and climacteric symptoms showed that postmenopausal or ovariectomised women with superficial dyspareunia had slightly, but significantly, lower mean plasma oestradiol, but not oestrone, concentrations when compared with levels in asymptomatic women. The hypothesis that superficial dyspareunia therefore resulted from a more severe degree of vaginal atrophy than occurred in asymptomatic women was supported by the finding in another group of women of a highly significant correlation of the karyopyknotic index and the random plasma

oestradiol, but not oestrone, concentration. If the biochemical concept of 'hormone replacement therapy' is the prescription of oestrogen to repair the circulating deficiency in symptomatic postmenopausal women, then the results of this study showed that the accurate fulfilment of this concept requires such therapy to increase the plasma oestradiol, but not oestrone, concentration in women with superficial dyspareunia to plasma levels about 3.8 ng/dl.

This study showed that the presence or timing of flushes was not related to a particular concentration of the plasma oestradiol, oestrone, androstenedione, FSH or LH concentration. Further studies of the role of plasma oestrogen and gonadotrophin concentrations during the climacteric in the pathogenesis of flushes are necessary so that more rational management of women with this distressing symptom can be developed.

Piperazine oestrone sulphate and oestradiol valerate are two pure oestrogen preparations currently prescribed for the treatment of climacteric symptoms that give rise, after absorption, to plasma oestrogens that supplement those produced endogenously. In this study the oral administration of oestradiol valerate and piperazine oestrone sulphate produced similar changes in the plasma concentrations of the unconjugated oestrogens in that any increase in the plasma oestradiol concentration was always accompanied by a preferential increase in the plasma oestrone levels. As parenteral administration of oestradiol increased the plasma oestradiol rather than the oestrone concentrations, the orally administered oestradiol must have been converted during absorption to oestrone. There were, however, considerable variations in the plasma oestrogen

concentrations after the administration of piperazine oestrone sulphate or oestradiol valerate, thus suggesting that different doses of these oral preparations may be required by different women to relieve climacteric symptoms. The treatment with piperazine oestrone sulphate or oestradiol valerate in the usually recommended doses did not fulfill either qualitative or quantitative concepts implicit in the term 'hormone replacement therapy' (as described above) as the plasma oestradiol levels in the women prescribed such therapy often exceeded the levels observed in asymptomatic postmenopausal women and occasionally also the levels seen during the mid-follicular phase of the ovulatory cycle in premenopausal women. Although the plasma oestrone concentrations in the women administered the oral oestrogen preparations were nearly always higher than seen at any other time of life, further studies are required to determine whether such unphysiological plasma levels of oestrone per se have pathological consequences.

Women treated with oestradiol implants are known to have a higher incidence of endometrial hyperplasia than women treated with oral therapy (Sturdee et al., 1978) but, unlike oral oestrogen therapy, there was not a preferential and marked increase in the plasma oestrone concentrations after an implant of oestradiol in the woman in this study. It is therefore unlikely that oestrone will prove to have a specific role in endometrial carcinogenesis, as hypothesised by Siiteri et al. (1974) and Ziel and Finkle (1976). This study suggested, however, that oestradiol was, biologically, the most important oestrogen circulating in women after the menopause. Further studies are necessary to determine whether

the development of endometrial hyperplasia is the inevitable consequence of proliferation resulting from levels of plasma oestrogens that are abnormal for postmenopausal women and the effects of which are not antagonised by progestogen.

Although the finding of a more significant fall of LH than FSH after the parenteral administration of oestradiol confirmed the findings of Nillius and Wide (1970) and Tsai and Yen (1971), the possible factors regulating the differential release of the gonadotrophins via the negative feedback axis were not clarified. Yen et al. (1975) found that there was a similar differential fall of gonadotrophin concentrations in postmenopausal women administered oestradiol orally but this was not confirmed in the present study.

ACKNOWLEDGEMENTS

This study would not have been possible without the enthusiastic collaboration of colleagues in the Departments of Obstetrics and Gynaecology and of Chemical Pathology at St. Mary's Hospital, to all of whom I am very grateful.

I am particularly indebted to Dr. Howard Jacobs for arousing my interest in this study three and a half years ago since when he has provided invaluable advice, especially in planning the study and then in preparing this manuscript. I am most grateful to Professor V.H.T. James for also supervising this study, and to Professor R.W. Beard for his support and encouragement during the last three years.

I owe a special debt of gratitude to Dr. Frances Murray for patiently teaching me the oestradiol radioimmunoassay, determining nearly all the oestrone and gonadotrophin concentrations, drawing all the figures and then, very kindly, typing this manuscript.

I would also like to thank Miss Ann Goodall, Mr. Peter Baxendale, Mr. Bernie Witchlow and Miss Beata Szitasi for determining all the plasma cortisol, androstenedione and testosterone concentrations, to Mr. John Fox for measuring some gonadotrophin concentrations and to Mrs Anne Morse for determining the karyopyknotic indices.

I would like to thank the consultants at the Samaritan Hospital for permission to study patients under their care, and the nursing staff of the Metabolic Ward at St. Mary's Hospital

and Dr. Norma Powell for assistance in collecting the blood samples. I am also very grateful to all the subjects who volunteered for the studies.

Finally, I would like to express my gratitude to Abbott Laboratories for an Abbott Research Fellowship and for additional financial support during the three years of the study.

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PLASMA OESTROGENS AFTER THE MENOPAUSE

Volume II

Appendices to thesis by

J.D. Hutton

CONTENTS

APPENDIX I. MATERIALS USED FOR BLOOD SAMPLING

Intravenous cannula: (Argyle Medicut) Outside diameter 1.3mm, length 45mm - Sherwood Medical Industries, London, U.K.

Three-way stopcock: K75 Luer, disposable ('Pharmoseal') - AHS/U.K. Ltd., Station Rd., Didcot, Oxford, U.K.

Syringes: 1,2,5 and 10ml. - Disposable ('Plastipak') - Becton, Dickinson and Company, Empire Way, Wembley, Middlesex, U.K.

Heparinised tubes: (capped) - 10ml, containing Lithium heparin - Searle Diagnostic, High Wycombe, Buckinghamshire, U.K.

Plain tubes: (capped) - 5ml - Searle Diagnostic, High Wycombe, Buckinghamshire, U.K.

Heparin injection: (Mucous) B.P., 5ml phial containing 5,000 iu/ml. Paines and Byrer Ltd., Greenford, Middlesex, U.K.

Saline: Sodium chloride B.P., 0.9% w/v; 50ml sterile solutions for injection prepared by the Pharmaceutical Department, St. Mary's Hospital, London, U.K.

Centrifuge: MSE Supermedium centrifuge - Measuring and Scientific Equipment Ltd., London, U.K.

Pasteur pipettes: Disposable capillary pipettes - length $5\frac{3}{4}$" - Harshaw Chemicals Ltd., Daventry, Northants.

APPENDIX II. MATERIALS AND DRUGS USED IN INFUSION AND CLINICAL STUDIES

Electrically-driven infusion pump: DN Infusion pump - Meltec Ltd., Bourne End, Buckinghamshire, U.K.

Anaesthesia extension set: (80cm), tubing capacity 2ml - Baxter laboratories Ltd., Thetford, Norfolk, U.K.

Scalp vein needle: ('Butterfly'), 21G (0.8mm) needle with tubing, Luer fitting - Abbott (Ireland) Ltd., Sligo, Republic of Ireland.

Syringe: 50ml disposable ('Plastipak') - Becton, Dickinson and company, Empire Way, Wembley, Middlesex, U.K.

Tetracosactrin: B.P. (Synacthen') - 0.25mg/ml aqueous solution - CIBA Laboratories, Horsham, Sussex, U.K.

Steroids: Androstenedione, testosterone, oestradiol, oestrone and cortisol purchased as dry products - Steraloids, Croyden, Surrey, U.K.

Sterile Actified Saline: Sodium chloride 8.76gm, water for injection 1L, dilute hydrochloric acid to adjust to pH 2. 50 ml bottles prepared - Pharmaceutical Department, St Mary's Hospital, London, U.K.

Ethanol: Research reagent distilled before use - James Burroughs Ltd., Montford Place, London, U.K.

Albumin: Human plasma protein fraction B.P. (Human albumin fraction - saline) for transfusion; 50ml sterile solutions for infusion containing 2.3gm protein - Blood Products Laboratory, Lister Institute, Elstree, Hertfordshire, U.K.

Saline: Sodium chloride B.P., 0.9% w/v, 50ml sterile solutions for injection - Pharmaceutical Department, St Mary's Hospital, London, U.K.

Drugs: Dexamethasone: ('Dexacortisyl') 0.5mg tablets - Roussel Laboratories Ltd., Wembley Park, Middlesex, U.K.

Piperazine oestrone sulphate: ('Harmogen'), 1.5mg tablets equivalent to 0.93mg oestrone - Abbott Laboratories Ltd., Queenborough, Kent, U.K.

Oestradiol valerate: ('Progynova') 1mg and 2mg tablets - Schering Chemicals Ltd., Burgess Hill, West Sussex, U.K.

Ethinyl oestradiol: ('Lynoral') 10µg tablets - Organon Laboratories Ltd., Crown House, London Rd., Morden, Surrey, U.K.

Oestradiol benzoate: 'Benztrone' 2mg/ml - Paines and Byrne, Greenford, Middlesex, U.K.

APPENDIX III. MATERIALS AND SPECIFIC TECHNIQUES USED IN OESTRADIOL RADIOIMMUNOASSAY

i) Chemicals (all were analytical grade and purchased from BDH Chemicals Ltd., Poole, Dorset, U.K., unless otherwise stated).

Methanol: was distilled before use.

Ethanol: James Burroughs Ltd., Montford Place, London, U.K. (absolute 99.8% v/v min.).

Distilled deionised water: using equipment from Demstill Equipment Ltd., Hove, Sussex, U.K., had zero conductivity, infinite resistance and a neutral pH.

Toluene:

Dichlorodimethylsilane: $(CH_3)_2$ $SiCl_2$ - MW 129.06 .

Decon 75: Medical Pharmaceuticals Developments Ltd., Brighton, Sussex, U.K.

PPO (2,5-diphenyloxazole) MW 221.26 - Fisons Scientific Apparatus, Loughborough, Leicestershire, U.K.

Dimethyl POPOP (1,4-tris-(2-methyl-5-phenyloxazolyl)-benzene), MW 392.46 - Fisons Scientific Apparatus, Loughborough, Leicestershire, U.K.

Norit-A activated charcoal: Sigma Chemicals,Ltd., Kingston-upon-Thames, Surrey, U.K.

Dextran T40: Pharmacia, Upsala, Sweden.

Diethyl-ether: (peroxide free) Fisons Scientific Apparatus, Loughborough, Leicestershire, U.K.

Gelatin powder:

Di-sodium hydrogen phosphate duodecahydrate: $(Na_2PO_4 \cdot 12H_2O)$ MW 358.16

Sodium dihydrogen orthophosphate: $(NaH_2PO_4 \cdot 2H_2O)$ MW 156.07

Sodium azide: (NaN_3) MW 65.01

Sodium chloride: (NaCl) MW 58.44

ii) Glassware and Pipettes

Glass test-tubes: Size B10 (6ml) were supplied by Quickfit and Quartz Ltd., Staffordshire, U.K.

Disposable glass test tubes: (rimless) in sizes 75 x 9.5 mm and 100 x 1515 mm were obtained from Scientific Supplies Ltd., London, U.K.

Repettes: were purchased from Jencons (Scientific) Ltd., Hemel Hempstead, Hertfordshire, U.K.

Pipettes: Semi-automatic pipettes for accurate measurements of volumes 10-1000µl were Marburg pipettes manufactured by Eppendorf Gerätebau, Germany and purchased from V.A. Howe Ltd., London, U.K.

All new glassware was cleaned by overnight soaking in chromic acid followed by rinsing next morning with tap water and a further soaking in 50% v/v aqueous methanol overnight. After this it was rinsed with tap water, distilled water and ethanol before being dried in an oven at 60°C. This initial treatment was followed, for the test tubes only, by a silanization process to coat the tubes and prevent any adsorption by the glass during assay work. This process consists of successive rinsing with 5% v/v solution of dichlorodimethylsilane in toluene, twice with toluene and twice with methanol, before heating to 110°C for two hours. All subsequent cleaning was carried out by soaking overnight in a 2% v/v solution of Decon 75 in distilled water. The next day, a rinsing procedure with tap water, distilled water and ethanol was carried out before drying in an oven at 60°C.

iii) Mixing, Shaking and Incubation: Gentle short term mixing was carried out using a rotamixer from Hook andTucker Ltd., London, U.K.

For more extensive shaking and for longer periods, a mechanical shaker (supplied by Townson and Mercer Ltd., Croyden, U.K.) was used. Cooling of the assay tubes, and then the addition and incubation of the charcoal suspension was carried out on a cold tray (supplied by Chemlab Instruments Ltd., Ilford, Essex, U.K.).

iv) Centrifugation: For general purposes, a MSE Supermedium centrifuge was used (obtained from Measuring and Scientific Equipment Ltd., London, U.K.). For refrigerated centrifugation a MSE 4L centrifuge was used.

v) Evaporation of Solvents: Ethereal and ethanolic solutions were evaporated using a dry heating block (Dri-block DB-3 Tecam Ltd., London, U.K.) in which the tubes were placed and over which oxygen-free nitrogen (British Oxygen Company Ltd., London, U.K.) was blown by means of a manifold and fine tubing. This was always performed in a fume cupboard with an extractor fan.

vi) Assay buffer: A phosphate buffer of pH 7.0 was first prepared as follows:-

In 2 litres of double deionised double distilled water were dissolved:-

43.7g di-sodium hydrogen phosphate duodecahydrate,
12.2g sodium dihydrogen orthophosphate,
2.0g sodium azide,
18.0g sodium chloride.

2.0g gelatin powder was then added to 2 litres of the above buffer, and heated gently until dissolved.

vii) Charcoal Suspension: In 100 ml of 0.1% gelatin phosphate buffer (above) were dissolved 0.625g Norit-A charcoal and 0.0625g dextran T40 with thorough mixing.

viii) Toluene scintillant: 3.0g PPO (as the primary phosphor solute) and 0.3g dimethyl POPOP (as the secondary solute) were dissolved in one litre of toluene. 10ml of this scintillant was dispensed into all the plastic counting phials (Griffiths and Nielsen, Essex U.K.) before the supernatant or recovery aliquots were added).

ix) Scintillation counting for tritium(^3H): These measurements were carried out using a Packard Tri-Carb Liquid Scintillation Spectrometer (Model Number 3380) obtained from Packard Instrument Co., U.K. office, Wembley, Middlesex, U.K. This had an efficiency of 35%.

x) Storage: Frozen samples were stored at -20°C until required for assay. Plasma pools were kept frozen in suitable aliquots for use in one assay only, thus avoiding repeated thawing and freezing. Solutions were stored in a refrigerator at 4°C. Other solutions were stored at room temperature.

APPENDIX IV

In this appendix the plasma hormone concentrations and clinical history are detailed for each of the subjects investigated. The age (in years), height (in centimetres) and weight (in kilogrammes) are those pertaining to the time of the first study. The '% Ideal Weight' is the difference between the subjects weight and the mean desirable weight for women of that height and having a medium frame, expressed as a percentage of the mean desirable weight (Geigy, 1970). The number of years since the menopause or ovariectomy is the interval between the menopause or ovariectomy and the time of the first study. The presence or absence of the climacteric symptoms of superficial dyspareunia and/or flushes where appropriate and the indications for, and response to oestrogen therapy are also detailed. When the subjects kept a record of the exact time of each flush during the sampling period, these times are given (in hours). The times of all hormone administrations during the sampling period are also detailed.

For clarity of presentation, the steroid hormones are abbreviated as follows. Cortisol = F, androstenedione = A, testosterone = T, oestradiol = E_2, oestrone = E_1. They are all measured in ng/dl except for cortisol which is measured in µg/dl and FSH and LH which are measured in µg/L.

Subject No. 1

Age: 54 Height: 160 cm Weight: 56 Kg % Ideal Weight + 5.6%

Years since menopause: 4.5

Symptoms: Flushes and superficial dyspareunia - local oestrogen treatment three months prior to study

Date of study: 16-17/8/76

Exact time of flushes: 0830; 0915; 1205; 1315; 1505; 1610; 1740; 1810; 1829; 1902; 1941; 2105; 2202; 2234; 2339; 0344; 0449; 0532; 0700 (hours)

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0800	4.0	3.0	22	109.3	13.5	21.1	696
0820	3.7	2.4	-	61.3	15.0	34.8	564
0840	3.9	3.6	31	56.0	13.0	40.7	559
0900	6.1	2.4	31	40.0	9.0	32.2	260
0920	5.2	1.8	23	56.0	7.5	33.3	662
0940	6.1	3.5	23	42.7	5.5	32.3	358
1000	7.4	3.3	31	56.0	4.5	37.2	480
1020	7.2	2.2	28	48.0	4.5	22.5	348
1040	7.4	4.6	23	40.0	7.0	17.2	216
1100	7.0	2.4	-	80.0	3.0	15.2	480
1120	7.0	1.3	29	106.7	6.5	25.5	451
1140	6.8	2.7	27	96.0	17.0	22.1	490
1200	6.5	1.2	27	105.3	9.0	24.0	696
1220	7.6	2.1	27	93.3	7.5	25.5	451
1240	6.5	2.5	30	149.3	7.5	23.5	431
1300	8.0	2.7	25	92.0	15.0	18.1	402
1320	7.5	2.8	25	93.3	25.5	27.4	549
1340	6.0	2.7	30	128.0	28.5	18.1	421
1400	5.4	3.9	23	53.3	25.0	23.5	500
1420	5.5	2.9	-	53.3	14.0	25.5	451
1440	7.3	2.8	18	45.3	9.0	22.1	451
1500	6.4	3.8	23	40.0	7.5	19.6	392
1520	6.6	3.4	22	45.3	8.5	29.4	529
1540	6.4	2.6	25	40.0	7.5	26.5	559
1600	6.2	2.2	30	45.3	4.5	25.5	421
1620	5.2	1.9	21	45.3	4.0	34.3	515
1640	4.5	2.4	18	68.0	10.0	27.9	319
1700	6.4	1.9	25	56.0	9.5	32.8	480
1720	6.4	-	28	56.0	6.0	41.2	421
1740	7.2	<1.0	-	50.7	5.0	42.1	461
1800	7.5	1.1	25	42.7	14.0	57.8	470
1820	7.0	1.7	25	48.0	5.0	38.2	431
1840	7.9	1.8	23	61.3	6.0	34.3	372
1900	5.6	<1.0	18	66.7	5.0	29.4	461
1920	4.7	"	25	73.3	7.5	22.5	451

subject no. 1 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
1940	5.8	<1.0	18	68.0	5.0	25.0	412
2000	6.5	1.6	25	28.0	4.0	20.1	309
2020	5.5	2.2	18	32.0	-	25.0	387
2040	6.9	2.2	21	32.0	6.5	18.1	461
2100	5.5	2.3	-	29.3	4.0	19.1	353
2120	6.0	2.3	18	45.3	3.5	21.6	461
2140	7.8	3.7	18	56.0	3.5	24.0	510
2200	5.9	2.5	21	28.0	3.0	18.6	470
2220	6.1	2.2	23	56.0	4.0	24.0	549
2240	6.7	1.6	35	58.7	5.0	17.6	402
2300	7.1	1.5	21	53.3	9.5	22.1	470
2320	5.6	<1.0	21	37,3	7.0	20.6	647
2340	4.0	1.5	23	52.0	3.5	21.7	539
2400	4.1	3.7	27	45.3	4.0	24.0	578
0020	3.2	<1.0	-	29.3	5.5	21.1	578
0040	5.5	"	27	74.7	9.0	25.0	323
0100	6.9	1.3	31	73.3	13.0	28.4	372
0120	6.6	1.5	35	72.0	9.5	24.5	412
0140	3.6	1.2	33	56.0	5.5	31.4	470
0200	3.9	-	28	40.0	9.5	36.3	338
0220	5.6	1.6	27	37.3	3.0	38.7	358
0240	7.2	1.2	25	48.0	10.0	38.6	279
0300	6.8	2.2	25	56.0	5.0	34.3	392
0320	7.4	2.2	28	73.3	16.0	26.0	348
0340	4.6	1.8	-	85.3	11.5	24.5	348
0400	7.2	3.4	21	45.3	13.0	22.1	431
0420	6.5	1.7	27	52.0	11.5	38.7	421
0440	5.6	3.5	27	85.3	14.5	22.5	382
0500	5.2	2.1	25	114.7	19.0	23.5	480
0520	6.2	2.8	25	112.0	9.5	26.5	490
0540	6.0	2.1	28	136.0	11.0	25.5	500
0600	7.9	1.0	18	101.3	14.5	21.6	549
0620	6.7	1.7	30	101.3	-	27.0	559
0640	6.8	1.6	23	93.3	-	24.5	539
0700	3.5	2.1	30	133.3	16.0	24.0	622
0720	6.8	1.7	30	98.7	15.5	21.6	662
0740	7.1	1.0	30	98.7	18.0	20.1	833
0800	6.2	1.0	-	85.3	-	21.1	784

Subject No. 2

Age: 51 Height: 162 cm Weight 53 Kg % Ideal Weight: - 2%

Years since menopause: 3.0

Symptoms: Flushes and superficial dyspareunia

Date of study: 17-18/10/76

Exact time of flushes: 0812; 0958; 1059; 1226; 1619; 1741; 1815; 2055; 2215; 0005; 0445; 0736 (hours)

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0800	5.2	2.9	64	213.3	27.0	-	-
0820	5.8	4.8	58	213.3	24.0	-	-
0840	5.8	4.5	55	141.3	21.5	33	500
0900	5.5	3.9	38	113.3	17.0	-	-
0920	5.7	2.6	39	100.0	15.5	34	450
0940	5.7	3.9	38	82.7	12.0	28	500
1000	4.4	3.5	45	73.3	9.0	32	490
1020	2.0	2.9	45	58.7	9.5	44	500
1040	4.9	4.2	52	58.7	9.0	32	506
1100	4.0	3.6	48	57.3	9.0	26	490
1120	3.8	2.1	55	81.3	7.0	21	530
1140	2.9	2.3	42	66.7	5.5	-	-
1200	2.2	2.9	43	77.3	5.5	30	505
1220	1.7	3.1	45	81.3	6.5	38	505
1240	1.7	4.2	64	154.7	19.5	39	595
1300	1.8	3.5	55	160.0	25.0	50	550
1320	3.3	2.9	64	165.3	31.5	41	475
1340	4.1	3.4	55	202.7	34.5	32	450
1400	4.7	3.3	52	208.0	35.5	33	550
1420	4.2	2.6	45	160.0	34.0	36	490
1440	4.3	2.0	52	176.0	30.0	31	510
1500	3.2	3.0	59	152.0	27.5	33	560
1520	2.3	3.1	48	104.0	20.5	46	580
1540	2.2	2.8	51	85.3	19.5	-	-
1600	2.3	3.2	35	56.0	17.0	32	500
1620	1.4	2.5	33	53.3	14.0	38	540
1640	1.9	3.4	37	84.0	12.5	39	575
1700	2.9	1.6	37	56.0	11.5	34	510
1720	4.5	2.5	28	64.0	10.5	27	490
1740	3.0	2.6	25	50.7	8.0	27	505
1800	4.2	2.2	52	65.3	8.5	36	525
1820	3.9	1.7	37	56.0	7.5	29	525
1840	3.3	2.2	35	57.3	6.0	38	530
1900	1.7	3.1	37	52.0	5.5	40	465
1920	2.8	4.5	45	61.3	4.0	40	490
1940	5.0	2.2	25	44.0	4.5	31	500

subject no. 2.(cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2000	4.7	3.0	45	44.0	4.0	33	510
2020	4.2	1.5	48	42.7	3.5	36	480
2040	4.2	2.9	55	40.0	3.5	31	490
2100	5.1	2.0	52	53.3	3.0	36	530
2120	5.6	1.7	52	56.0	3.0	43	515
2140	4.8	1.4	40	50.7	3.0	29	550
2200	4.7	2.9	44	45.3	2.5	30	470
2220	4.8	2.0	38	26.7	1.5	42	530
2240	5.3	1.7	43	25.3	2.5	41	555
2300	5.7	1.5	41	50.7	3.0	25	550
2320	4.9	3.0	37	42.7	3.0	40	550
2340	5.6	2.0	52	65.3	3.0	35	475
2400	4.5	2.4	49	42.7	3.0	29	510
0020	5.1	1.6	45	45.3	2.5	41	530
0040	5.1	2.5	39	42.7	2.5	45	490
0100	3.9	4.1	41	34.7	3.0	48	530
0120	5.4	1.9	35	58.7	3.5	38	555
0140	4.3	2.7	49	32.0	3.0	38	555
0200	4.7	1.4	47	50.7	2.0	38	485
0220	5.7	1.9	35	60.0	3.0	38	550
0240	5.3	1.3	38	117.3	9.5	33	500
0300	4.7	1.4	47	80.0	6.5	20	400
0320	5.1	1.9	52	118.7	11.5	38	545
0340	5.6	2.6	52	170.7	17.5	33	455
0400	5.9	2.3	41	128.0	14.5	35	480
0420	6.1	<1.0	45	98.7	12.0	30	530
0440	6.2	1.4	37	92.0	9.0	33	525
0500	5.6	2.5	45	224.0	18.0	42	465
0520	5.5	3.0	40	194.7	18.5	36	510
0540	4.3	1.5	40	90.7	13.5	34	490
0600	5.0	1.8	32	81.3	10.5	40	510
0620	5.3	<1.0	49	77.3	9.5	36	520
0640	4.8	1.5	50	80.0	8.5	31	520
0700	4.7	<1.0	52	144.0	13.0	25	395
0720	4.9	1.5	54	178.7	17.5	-	-
0740	4.0	1.6	52	213.3	-	-	-
0800	3.9	3.8	37	208.0	25.0	-	-

Subject No. 3

Age: 51 Height: 163 cm Weight 56 Kg % Ideal Weight + 2.5%

Years since menopause: 4.0

Symptoms: Neither flushes nor superficial dyspareunia

Date of study: 14-15/1/76

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0800	2.4	4.6	30	98.7	-	36.3	735
0820	7.8	6.0	26	85.3	13.5	31.4	647
0840	4.8	4.9	28	85.3	12.0	34.8	549
0900	5.3	3.9	25	58.7	11.5	30.4	696
0920	5.0	4.0	22	61.3	10.0	30.4	564
0940	2.7	3.4	22	48.0	7.0	30.4	725
1000	3.5	1.7	22	48.0	7.0	33.8	564
1020	4.7	1.5	18	52.0	6.0	29.4	647
1040	5.0	2.8	20	53.3	7.0	31.4	686
1100	4.3	2.0	20	41.3	2.5	37.7	539
1120	3.7	1.0	26	50.7	4.0	30.9	676
1140	3.4	1.8	28	56.0	4.5	39.2	970
1200	5.3	6.4	25	57.3	5.0	41.2	696
1220	5.2	1.5	26	48.0	7.0	39.2	686
1240	4.0	1.0	25	61.3	7.5	43.1	725
1300	4.5	1.6	25	72.0	7.0	40.2	813
1320	3.0	2.3	26	77.3	5.5	44.1	431
1340	1.3	3.0	26	72.0	6.5	51.5	470
1400	5.5	3.2	20	74.7	6.0	58.8	500
1420	4.4	1.8	25	69.3	7.5	46.1	461
1440	3.1	3.4	31	74.7	6.0	44.1	549
1500	1.5	3.2	30	73.3	7.0	53.9	515
1520	3.5	2.8	28	58.7	6.5	36.3	461
1540	4.3	2.2	25	69.3	5.5	32.3	480
1600	3.9	2.3	25	64.0	5.5	30.4	510
1620	2.6	1.7	26	82.7	7.0	30.4	539
1640	5.4	1.1	25	73.3	7.0	33.8	549
1700	4.1	2.1	20	77.3	7.0	36.3	559
1720	5.6	5.4	26	88.0	-	39.2	696
1740	5.0	4.4	20	88.0	-	35.3	662
1800	3.8	2.8	26	72.0	-	32.3	735
1820	1.9	2.6	31	74.7	10.0	31.4	578
1840	2.8	7.0	26	46.7	7.5	21.1	662
1900	3.2	4.0	28	53.3	6.5	28.4	784
1920	1.8	4.8	26	66.7	5.0	31.4	696
1940	2.7	3.9	20	41.3	7.5	36.3	882
2000	7.2	5.3	25	46.7	5.0	42.1	872
2020	3.8	4.1	25	40.0	4.0	39.2	813
2040	3.7	5.6	25	41.3	3.5	37.2	564

subject no. 3 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2100	6.2	4.8	20	40.0	3.0	49.0	725
2120	3.2	5.6	28	34.7	3.5	46.1	735
2140	1.7	4.4	22	41.3	3.0	34.3	696
2200	3.2	3.6	30	29.3	2.5	58.8	725
2220	5.1	3.0	25	48.0	4.0	46.1	686
2240	4.2	3.0	26	53.3	3.0	46.3	907
2300	2.8	2.3	26	42.7	2.5	31.9	676
2320	2.6	2.0	25	42.7	2.0	36.3	735
2340	2.9	2.4	25	37.3	2.5	34.3	774
2400	5.3	1.9	26	26.7	-	38.2	662
0020	1.7	3.4	22	41.3	-	27.9	686
0040	1.6	-	25	-	-	37.2	715
0100	3.3	2.3	28	46.7	-	33.3	715
0120	3.7	3.0	22	40.0	-	30.4	622
0140	2.6	3.1	25	26.6	-	-	-
0200	2.9	4.5	26	61.3	2.5	32.8	784
0220	8.6	5.9	22	85.3	5.0	31.9	774
0240	9.2	2.8	25	56.0	3.5	41.2	813
0300	7.8	3.0	25	80.0	-	-	431
0320	8.7	3.6	30	98.7	16.0	51.5	833
0340	7.2	4.3	30	85.3	12.5	56.4	980
0400	6.0	2.2	30	58.7	12.5	52.9	1024
0420	6.8	2.5	31	58.7	8.0	41.2	956
0440	6.7	4.1	35	66.7	-	-	431
0500	4.1	1.0	33	48.0	-	46.1	956
0520	4.9	2.3	31	61.3	6.5	49.6	-
0540	5.6	2.6	35	92.0	9.5	46.6	956
0600	7.5	5.3	37	114.7	-	56.4	676
0620	8.6	3.5	33	104.0	13.0	68.6	662
0640	7.8	4.4	30	90.7	17.0	39.2	686
0700	8.9	4.7	30	100.0	14.5	68.6	539
0720	7.6	4.1	28	106.7	18.5	68.6	715
0740	4.6	5.5	33	88.0	16.0	66.2	461
0800	1.0	4.5	22	96.0	14.5	58.8	676

Subject No.4

Age: 55 Height: 172 cm Weight: 69 Kg % Ideal Weight + 12%

Years since menopause: 5

Symptoms: None - referred to gynaecological clinic because of abnormal smear detected during routine screening. Incomplete excision of carcinoma-in-situ lesion of the cervix by cone biopsy 25/11/75

Date of study: 10-11/2/75

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0820	6.8	7.0	-	-	10.0	20.6	539
0840	8.2	5.7	48	165.3	16.0	21.1	451
0900	8.1	5.5	57	-	-	22.1	255
0920	4.6	5.5	54	104.0	11.0	23.0	333
0940	7.1	4.6	41	77.3	11.0	21.6	201
1000	5.4	4.2	44	72.0	-	21.6	333
1020	8.0	5.9	44	66.7	7.0	20.6	333
1040	4.7	4.2	54	-	-	21.1	304
1100	5.8	3.8	54	104.0	3.0	21.1	392
1120	7.3	7.8	44	68.0	3.0	14.2	289
1140	10.7	5.6	44	66.7	4.0	20.6	451
1200	7.8	5.3	44	66.7	5.0	22.1	304
1220	5.9	4.4	48	96.0	5.0	17.2	372
1240	6.2	5.7	45	117.3	6.0	17.6	333
1300	9.1	4.9	45	149.3	8.0	22.5	274
1320	5.2	5.9	37	125.3	8.0	17.2	490
1340	8.5	4.9	44	136.0	10.0	18.1	588
1400	4.8	4.9	44	-	7.0	23.0	245
1420	1.6	6.5	45	157.3	11.0	28.9	421
1440	4.9	6.6	44	144.0	12.0	23.5	314
1500	7.1	5.3	48	144.0	9.0	32.9	564
1520	5.6	5.6	49	176.0	9.0	35.8	211
1540	9.5	6.2	57	-	-	19.6	588
1600	10.1	6.1	48	157.3	6.0	30.4	578
1620	7.8	6.5	51	152.0	6.0	37.2	250
1640	7.6	6.0	51	165.3	6.0	27.4	314
1700	5.2	6.6	32	-	6.0	25.5	431
1720	6.8	8.3	48	-	5.0	21.1	364
1740	5.8	5.1	33	120.0	4.0	22.5	392
1800	8.7	4.4	47	-	3.5	22.5	364
1820	9.4	4.6	47	97.3	-	18.6	402
1840	8.2	6.7	41	-	-	19.6	382
1900	9.8	3.2	41	144.0	3.0	21.1	539
1920	10.1	6.8	41	112.0	3.5	22.5	461
1940	4.5	6.5	35	114.7	3.5	17.6	412
2000	5.9	4.2	35	85.3	-	21.1	392

subject no. 4 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2020	5.7	4.5	49	102.7	4.0	22.5	348
2040	6.3	5.5	-	-	-	-	-
2100	7.4	5.1	49	176.0	7.5	23.5	515
2120	9.8	3.5	41	106.7	6.5	24.0	637
2140	6.9	3.8	47	112.0	11.0	23.0	510
2200	9.0	4.7	44	105.3	7.0	19.1	564
2220	6.3	5.4	41	154.7	9.0	27.4	333
2240	6.2	4.3	55	125.3	8.0	23.5	505
2300	9.7	5.8	56	144.0	9.0	25.5	303
2320	9.1	5.1	55	-	2.5	-	-
2340	8.4	5.9	57	90.7	4.0	26.5	353
2400	10.6	5.6	51	-	6.0	31.4	412
0020	8.8	2.6	52	90.7	4.0	32.3	519
0040	7.5	4.6	49	68.0	3.0	30.4	348
0100	9.9	7.0	58	88.0	2.5	25.0	402
0120	8.9	7.0	55	77.3	"	22.1	265
0140	9.6	6.6	57	90.7	"	28.4	676
0200	8.6	5.8	62	170.7	6.0	27.4	353
0220	4.6	7.6	60	98.0	8.0	22.5	568
0240	6.1	3.9	53	105.3	7.0	22.1	333
0300	6.9	8.1	39	120.0	7.0	20.6	348
0320	4.6	4.4	51	-	7.0	-	441
0340	8.8	4.5	52	-	5.0	24.5	402
0400	9.4	4.6	42	-	4.0	22.1	319
0420	9.7	7.3	50	102.7	10.0	24.0	421
0440	10.2	7.2	55	178.7	20.0	21.1	539
0500	8.4	4.6	48	181.3	13.0	21.1	539
0520	8.5	4.2	47	122.7	13.0	26.5	451
0540	7.2	5.3	50	106.7	10.0	19.1	578
0600	8.1	4.8	55	130.7	14.0	27.9	461
0620	7.3	5.5	43	186.7	15.0	27.4	480
0640	8.3	5.4	43	-	15.0	30.4	637
0700	4.0	5.6	50	149.3	13.0	28.9	588
0720	3.8	6.0	50	-	12.0	-	662
0740	3.0	6.4	48	125.3	9.0	24.5	539
0800	5.9	6.6	-	-	9.0	26.5	760
0820	7.4	4.0	-	-	9.0	23.0	613

Subsequent management: 12/2/76 total abdominal hysterectomy & bilateral ovariectomy: histologically carcinoma-in-situ of the cervix apparent, but no primordial follicles identified in either ovary.

Subject No. 5

Age: 55 years Height: 160 cm Weight: 54Kg % Ideal Weight: + 2.6%

Years since menopause: 4.5

Symptoms: Both flushes and superficial dyspareunia

Date of first study: 28-29/12/75

Exact time of flushes: 0755; 0930; 1038; 1112; 1138; 1303; 1350; 1450; 1536; 1619; 1723; 1810; 1838; 1935; 2048; 2137; 2219; 2258; 0654; 0746 (hours)

Times asleep: 2300-0320; 0420-0520 (hours)

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0800	1.0	4.3	-	No Samples		8.5	400
0820	-	-	-			7.2	270
0840	1.1	3.2	-			3.6	240
0900	1.0	5.6	-			10.0	210
0920	1.0	1.0	-			10.0	280
0940	1.3	1.1	45			10.0	220
1000	1.4	2.7	-			3.8	560
1020	2.9	4.3	-			6.6	370
1040	4.8	1.1	-			6.0	245
1100	3.1	1.1	-			7.3	370
1120	2.2	1.1	-			6.0	245
1140	1.0	1.3	43			7.4	265
1200	1.0	1.0	37			10.0	205
1220	1.0	1.0	-			3.7	265
1240	1.0	1.0	42			6.7	280
1300	1.0	1.0	-			9.0	430
1320	1.0	1.0	-			6.0	240
1340	2.8	1.0	42			9.5	200
1400	1.6	1.0	-			10.0	195
1420	2.6	1.0	-			12.0	220
1440	2.6	1.0	-			6.0	240
1500	2.8	1.0	-			11.5	315
1520	2.8	1.0	40			10.0	240
1540	1.9	1.0	40			10.0	210
1600	1.4	1.0	48			6.2	250
1620	2.0	1.0	34			2.3	360
1640	1.5	1.0	28			5.6	280
1700	1.4	1.0	-			10.5	500
1720	2.5	1.0	-			10.0	225
1740	4.4	1.2	38			14.0	195
1800	1.8	1.3	-			8.1	160
1820	3.5	1.0	55			2.1	280
1840	4.2	2.0	54			6.7	255

subject no. 5 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
1900	3.1	1.3	43	No	Samples	5.4	185
1920	1.0	1.0	70			7.9	220
1940	2.3	1.2	42			4.9	195
2000	1.9	1.0	-			3.0	210
2020	1.1	1.0	55			8.0	255
2040	2.6	1.0	-			6.0	215
2100	3.6	1.0	38			2.8	195
2120	9.7	1.0	33			3.3	200
2140	9.2	1.0	51			3.6	235
2200	4.1	1.0	51			8.2	180
2220	2.5	1.0	55			4.4	175
2240	4.5	1.0	68			5.2	160
2300	3.6	1.0	42			5.6	180
2320	3.4	1.0	-			10.0	190
2340	3.4	1.0	45			9.0	240
2400	2.5	5.1	-			4.8	180
0020	3.3	1.0	45			8.0	205
0040	3.0	1.0	40			4.3	250
0100	3.4	1.0	34			3.9	275
0120	2.1	1.0	-			2.3	270
0140	2.8	1.0	-			3.4	225
0200	4.1	1.0	-			6.0	280
0220	4.5	1.0	38			9.2	195
0240	7.4	6.3	51			7.3	200
0300	5.7	8.7	45			3.4	265
0320	6.0	1.0	43			4.8	220
0340	9.2	1.2	38			7.5	200
0400	6.8	1.0	68			10.0	190
0420	2.3	1.0	55			10.0	220
0440	3.7	1.0	79			3.9	240
0500	3.8	1.0	-			8.0	270
0520	4.3	2.9	-			10.0	255
0540	3.8	1.2	-			5.4	180
0600	2.2	1.6	50			4.4	166
0620	4.5	3.1	-			3.8	180
0640	4.6	1.0	-			2.8	230
0700	3.9	5.4	-			3.5	275
0720	2.5	1.0	50			6.3	295
0740	3.6	1.0	-			4.0	300
0800	-	6.2	-			2.7	260

Date of second study: 25-26/6/76 - first day of new treatment cycle

Symptoms: Experiencing some relief of flushes and superficial dyspareunia with Harmogen 1.5mg (prescribed cyclically).

Previous years of oestrogen therapy: 0.5

(No improvement in climacteric symptoms nor withdrawal bleeding in the treatment cycle subsequent to the study)

subject no. 5 second study - hormone concentrations.

Time	E_1	E_2	T	A	F	LH	FSH
1000	5.4	2.7	20	69.3	7.5	18.6	686
1030	4.3	2.7	23	46.7	5.5	24.0	696
1100	4.2	3.2	28	50.7	4.5	21.6	735
1130	5.0	1.8.	28	61.3	4.5	27.4	627
1200	2.7	1.6	29	90.7	7.5	26.5	725
1230	3.3	2.3	28	66.7	6.0	27.4	833
1300	2.6	3.2	28	50.7	5.0	16.7	907
1330	2.8	1.9	24	66.7	5.5	22.1	745
1400	3.0	2.4	29	120.0	10.5	27.0	784
1430	3.5	2.0	29	110.7	9.5	24.0	515
1500	4.0	2.5	24	114.4	9.0	22.5	559
1530	4.6	2.4	30	63.8	7.5	27.4	500
1600	5.5	2.4	24	59.9	<2.5	29.4	431
1630	12.3	3.5	28	37.2	6.0	33.8	559
1700	11.5	6.1	22	39.9	4.0	29.9	470
1730	11.7	5.5	24	31.9	3.0	41.2	490
1800	14.2	6.6	28	31.9	<2.5	38.2	470
1830	25.9	5.5	23	26.6	<2.5	44.1	578
1900	25.8	6.3	28	50.5	7.5	24.0	470
1930	36.6	6.1	30	54.5	5.0	31.4	461
2000	18.8	6.0	28	58.5	6.5	27.4	500
2030	14.2	5.0	22	39.9	3.5	23.0	500
2100	19.9	6.0	28	37.2	2.5	24.0	549
2130	15.3	6.1	28	41.2	4.5	28.4	490
2200	16.3	4.2	29	69.2	5.5	24.5	510
2230	12.8	4.1	28	72.0	7.0	28.9	500
2300	8.8	3.7	28	42.7	5.0	24.0	539
2330	10.7	4.0	28	49.3	7.5	27.0	559
2400	9.1	3.0	24	90.7	7.5	26.5	622
0030	8.4	3.2	25	45.3	3.5	20.1	549
0100	8.5	1.7	31	93.3	4.5	19.1	431
0130	6.5	3.0	28	53.3	4.0	24.0	323
0200	9.0	2.4	28	80.0	5.0	24.0	372
0230	4.8	1.6	32	66.7	4.0	21.6	392
0300	5.0	1.5	30	28.0	3.5	21.6	412
0330	8.5	1.5	30	66.7	3.0	19.1	421
0400	7.8	<1.0	31	77.3	4.0	27.0	461
0430	7.3	3.0	31	64.0	6.5	37.2	431
0500	NO SAMPLE			-	-	-	-
0530	8.8	3.0	28	149.3	10.0	33.8	412
0600	5.2	3.2	32	93.3	12.0	31.9	451
0630	4.6	3.0	35	106.7	10.5	49.0	480
0700	8.6	3.1	31	106.7	14.5	29.4	500
0730	9.6	2.7	31	80.0	11.0	24.0	451
0800	7.6	<1.0	30	50.7	9.5	23.0	461
0830	4.2	<1.0	35	53.3	7.5	22.5	480
0900	5.9	<1.0	29	42.7	5.5	24.0	529
0930	6.9	<1.0	36	-	-	27.4	451
1000	6.1	<1.0	37	74.6	9.5	20.1	382

Subject No. 6

Age: 54 Height: 161 cm Weight: 61 Kg % Ideal Weight + 12%

Years since menopause: 1.9

Symptoms: Neither flushes nor superficial dyspareunia

Date of study: 9-11/2/77
Dexamethasone treatment: 2mg 2400h 10/2/77,
0.5mg 0800h & 1600h 11/2/77
Tetracosactrin infusion: 0830-1430h 11/2/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	7.1	4.4	78	-	7.5	No Samples	
0030	7.0	4.0	62	98.7	7.2		
0100	7.3	4.1	56	101.3	6.6		
0130	7.8	3.3	53	77.3	6.4		
0200	8.6	4.7	50	72.0	4.4		
0230	5.1	4.2	55	92.0	5.2		
0300	3.9	4.9	52	82.7	7.6		
0330	4.4	2.7	57	109.3	13.6		
0400	6.9	2.6	62	154.7	15.8		
0430	8.2	4.0	70	138.7	12.1		
0500	7.5	3.8	53	133.3	15.6		
0530	5.5	4.9	60	232.0	18.0		
0600	3.8	4.0	62	200.0	23.2		
0630	2.4	5.5	72	232.0	23.4		
0700	5.2	6.7	62	165.3	19.0		
0730	5.9	5.1	84	240.0	29.7		
0800	6.2	5.2	68	256.0	24.8		
0830	7.2	5.7	62	114.7	21.5		
0900	6.7	4.9	66	117.3	18.8		
0930	6.6	5.3	58	120.0	16.9		
1000	5.5	4.8	70	112.0	13.8		
1030	6.0	5.0	57	109.3	14.4		
1100	6.9	5.0	53	125.3	15.3		
1130	6.6	4.6	52	178.7	15.0		
1200	7.9	5.3	72	144.0	24.7		
2400	8.2	3.9	46	133.3	17.4		
0030	7.7	3.9	52	102.7	12.4		
0100	7.0	3.3	46	61.3	7.0		
0130	7.3	3.9	50	62.7	3.9		
0200	7.3	4.5	43	58.7	6.2		
0230	6.1	5.0	48	61.3	5.1		
0300	6.0	4.8	43	62.7	7.2		
0330	6.9	4.8	40	45.3	4.6		
0400	5.3	4.8	40	48.0	4.6		
0430	4.5	4.2	45	32.0	2.9		
0500	< 1.0	4.0	46	29.3	2.7		

subject no. 6 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0530	<1.0	4.4	43	40.0	1.8	No Samples	
0600	3.2	4.7	38	32.0	1.1		
0630	<1.0	4.7	45	41.3	2.0		
0700	2.5	4.8	51	29.3	1.1		
0730	2.5	4.0	47	45.3	1.6		
0800	2.3	4.2	47	56.0	2.6		
0830	4.4	3.8	30	34.7	1.9		
0900	6.8	3.7	47	240.0	22.8		
0930	8.6	4.7	58	320.0	26.4		
1000	6.4	2.4	78	333.3	34.3		
1030	7.3	3.5	82	346.7	34.0		
1100	10.1	3.9	80	266.7	32.5		
1130	9.8	6.9	70	293.3	31.5		
1200	12.8	5.7	82	306.7	34.7		
1230	10.0	6.2	84	386.7	35.6		
1300	11.9	5.0	-	293.3	34.2		
1330	11.0	7.9	90	280.0	35.0		
1400	9.3	5.1	90	229.3	36.5		
1430	9.1	7.2	80	274.7	35.9		
1500	8.3	6.8	84	293.3	33.5		
1530	7.7	5.5	74	226.7	27.8		
1600	8.5	6.8	73	226.7	30.8		
1630	8.0	4.5	64	128.0	25.9		
1700	6.9	5.1	72	112.0	24.4		
1730	7.6	4.3	66	154.7	23.4		
1800	6.9	4.4	60	106.7	19.8		
1830	6.5	5.0	62	186.7	14.1		

Subject No. 7

Age: 53 Height: 173 cm Weight: 63 Kg % Ideal Weight: + 1%

Years since menopause: 3.0

Symptoms: Flushes but no superficial dyspareunia

Date of first study: 2-3/6/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	4.9	3.6	23	56.0	8.1	No Samples	
0030	4.9	3.9	25	57.3	9.5		
0100	5.2	5.4	31	61.3	5.2		
0130	5.3	4.7	28	57.3	3.3		
0200	5.0	4.6	23	49.3	4.6		
0230	5.2	3.4	22	68.0	2.6		
0300	5.3	3.8	20	45.3	9.3		
0330	4.2	4.0	24	66.7	8.1		
0400	6.1	3.1	18	38.7	12.6		
0430	4.7	4.2	17	36.0	11.0		
0500	5.7	3.7	20	64.0	17.7		
0530	4.8	2.5	23	100.0	18.0		
0600	4.4	2.9	19	40.0	14.4		
0630	5.1	3.4	27	60.0	21.5		
0700	4.3	3.6	28	58.7	18.0		
0730	4.4	2.9	26	73.3	20.7		
0800	4.5	2.7	14	72.0	19.5		
0830	1.4	4.0	17	58.7	20.8		
0900	4.7	4.3	14	49.3	15.1		
0930	NO	SAMPLE		-	-		
1000	2.6	2.8	15	<26.6	10.6		
1030	5.5	3.0	17	"	6.9		
1100	4.1	1.5	13	"	6.5		
1130	6.3	3.1	14	"	5.8		
1200	4.4	1.9	26	78.7	11.9		

Date of second study: 10-11/6/77
Dexamethasone treatment: 1mg 2300h & 0.5mg 0800h prescribed on discharge after first study, & continued during second study
Tetracosactrin infusion: 0830-1430h 11/6/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	3.2	1.8	<10	<26.6	4.3	No Samples	
0030	2.8	1.8	17	"	1.8		
0100	2.9	1.5	17	"	<1.0		
0130	3.1	1.6	21	"	"		

subject no. 7 second study (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0200	2.9	<1.0	20	<26.6	<1.0	No Samples	
0230	2.7	"	13	"	"		
0300	2.6	1.5	13	"	"		
0330	2.7	1.4	<10	"	"		
0400	2.3	2.2	"	33.3	"		
0430	2.3	<1.0	"	<26.6	"		
0500	2.5	"	14	"	"		
0530	3.3	1.7	11	"	"		
0600	3.1	<1.0	10	"	"		
0630	3.3	2.6	12	"	"		
0700	3.1	2.6	10	"	"		
0730	3.4	<1.0	10	"	"		
0800	2.9	2.5	<10	"	"		
0830	2.1	1.8	12	"	"		
0900	3.0	<1.0	<10	"	7.3		
0930	3.0	1.2	13	54.7	11.3		
1000	3.4	1.1	10	52.0	13.7		
1030	2.7	<1.0	13	41.3	17.8		
1100	3.3	"	12	44.0	18.5		
1130	3.8	1.1	20	40.0	18.4		
1200	5.7	1.0	12	60.0	18.5		
1230	4.0	1.3	15	52.0	19.3		
1300	2.5	1.2	14	60.0	19.8		
1330	5.2	1.7	12	73.3	19.3		
1400	4.8	1.6	<10	52.0	19.6		
1430	4.4	1.4	10	49.3	19.6		
1500	2.3	2.7	10	66.9	18.3		
1530	3.1	1.9	14	40.0	15.6		
1600	4.5	2.7	11	34.7	12.9		
1630	4.5	<1.0	14	28.0	10.1		
1700	5.7	2.0	22	40.0	11.1		
1730	5.7	-	17	48.0	7.6		
1800	6.1	<1.0	<10	<26.6	6.0		
1830	3.7	1.3	17	"	4.0		

Subject No. 8

Age:51 Height: 153 cm Weight: 57 Kg % Ideal Weight: + 11.6%

Years since menopause: 0.4

Symptoms: Flushes but no superficial dyspareunia

Date of study: 16-17/1/76

Time of flushes: 0821; 1859; 1035; 1215; 1450; 1530; 1745; 1858; 2035; 2139; 2210; 2250; 2321; 0005; 0141; 0410; 0450; 0530; 0611; 0651; 0806 (hours)

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0800	10.6	4.2	45	N.S.	-	-	-
0820	9.7	4.8	-		13.0	26.5	402
0840	14.2	3.9	-		16.0	28.9	470
0900	14.5	2.6	45		-	-	-
0920	15.9	5.1	62		12.0	-	-
0940	4.5	4.6	-		12.0	18.6	274
1000	6.7	2.6	48		-	-	-
1020	6.6	2.9	-		12.0	21.6	245
1040	3.5	2.4	45		8.5	26.5	216
1100	4.4	2.9	45		7.0	26.0	157
1120	4.6	3.4	49		5.5	24.0	221
1140	5.9	4.4	45		8.0	-	-
1200	7.1	6.4	48		10.5	-	-
1220	8.1	1.5	48		15.0	32.8	196
1240	8.8	2.7	52		13.0	37.2	245
1300	10.5	1.4	49		10.5	-	-
1320	9.9	4.7	41		7.5	-	-
1340	8.0	3.3	-		-	-	-
1400	9.7	3.8	-		-	-	-
1420	16.0	4.3	45		7.5	-	-
1440	13.5	3.6	43		5.0	32.0	279
1500	11.1	4.2	48		5.5	32.0	274
1520	15.1	6.6	43		4.5	-	-
1540	4.1	6.5	42		9.5	32.3	230
1600	2.8	2.0	42		12.0	-	-
1620	4.8	5.1	41		11.5	29.9	255
1640	3.5	4.9	49		11.0	-	-
1700	3.1	3.6	48		9.0	27.9	480
1720	4.8	4.3	48		8.0	-	-
1740	10.1	4.7	50		6.5	28.9	260
1800	3.8	7.2	45		6.5	31.9	255
1820	2.8	2.0	43		-	-	-
1840	1.9	1.9	45		4.0	-	-
1900	1.4	4.8	38		3.5	-	-
1920	5.9	2.3	-		3.5	29.4	221
1940	3.5	1.7	-		3.0	31.4	172

subject no. 8 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2000	5.2	5.0	-	N.S.	2.5	22.1	245
2020	6.2	4.1	-		6.0	30.9	216
2040	3.1	4.3	-		4.0	32.3	235
2100	5.8	2.9	-		3.5	32.3	221
2120	3.1	5.6	-		3.5	31.4	216
2140	2.6	2.0	34		3.0	-	-
2200	5.7	4.2	38		2.5	32.8	235
2220	5.3	3.7	-		2.0	-	-
2240	8.5	2.7	-		2.0	31.4	235
2300	3.9	2.7	38		-	31.4	-
2320	5.9	4.5	-		2.0	32.3	245
2340	6.5	4.3	45		-	-	-
2400	6.7	3.4	-		2.0	32.8	216
0020	8.0	2.8	-		1.0	26.5	235
0040	8.1	2.4	-		1.0	26.5	255
0100	4.9	-	-		2.0	24.5	314
0120	10.0	2.0	-		6.4	-	-
0140	5.6	2.2	-		9.9	-	-
0200	4.8	5.5	-		13.2	22.1	412
0220	7.2	2.0	-		9.0	-	-
0240	9.2	4.3	-		6.0	27.0	451
0300	5.0	6.6	-		6.3	-	-
0320	8.6	4.4	-		4.0	34.8	372
0340	8.9	2.5	-		-	-	-
0400	6.5	3.9	-		3.5	-	-
0420	9.7	5.0	-		7.6	29.4	206
0440	10.9	2.6	43		3.6	-	-
0500	4.9	5.7	-		5.6	-	-
0520	7.4	5.7	-		-	-	-
0540	11.8	-	-		8.0	31.4	245
0600	10.4	3.6	52		-	19.1	206
0620	9.8	2.9	61		-	-	-
0640	6.4	3.1	-		11.5	25.5	235
0700	6.6	4.9	-		13.5	29.4	289
0720	10.3	4.1	53		-	-	-
0740	12.6	3.8	-		13.0	-	-
0800	6.7	-	-		15.0	24.0	299

Subsequent History: Commenced on Harmogen 1.5mg cyclically 17/1/76, but discontinued 28/5/76 because of regular withdrawal bleeding and relief of flushes. Had periods on 4/4/76, 4/5/76, 6/6/76, 6/7/76, 8/8/76 & 1/10/76. Oestrogen therapy was recommenced on 29/11/76 because of a recurrence of flushes. Plasma E_2, LH & FSH levels in random blood samples during this time were:-

Date	E_2	LH	FSH
16/1/76	3.8	28.7	268
24/5/76	12.0	3.2	210
28/6/76	3.1	8.2	72
26/7/76	15.3	50.0	240
23/8/76	4.5	6.4	80
11/10/76	13.1	4.5	32
20/10/76	3.2	3.2	29
29/11/76	3.0	17.5	210

Subject No.9

Age: 48 Height: 165 cm Weight: 56 Kg % Ideal Weight: + 3.7%

Years since menopause: 0.4

Symptoms: Flushes and superficial dyspareunia

Date of study: 3-4/8/76
Oestrogen therapy: Piperazine oestrone sulphate (3mg) prescribed 2300h 3/8/76. Subsequent relief of symptoms during treatment cycle, but no withdrawal bleeding.

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1830	5.4	2.9	28	<26.6	7.5	34.3	603
1900	4.6	1.0	28	28.0	13.5	57.8	627
1930	7.3	<1.0	24	<26.6	7.0	53.9	676
2000	5.1	"	24	"	6.0	37.2	529
2030	7.2	"	26	34.7	6.0	39.2	461
2100	7.6	2.7	28	26.6	4.5	34.3	490
2130	9.2	1.0	34	52.0	4.0	41.2	500
2200	8.3	3.4	28	57.3	4.0	41.2	725
2230	8.1	<1.0	25	-	2.5	27.0	372
2300	12.9	"	No	Samples	2.5	29.4	358
2330	14.1	"			2.5	41.2	515
2400	17.5	"			2.5	36.3	529
0030	12.4	3.2			2.5	28.4	539
0100	18.6	5.0			2.5	33.3	461
0130	27.4	2.7			2.5	41.2	480
0200	23.7	1.0			2.5	36.3	451
0230	18.8	1.4			3.0	36.3	392
0300	24.4	3.2			2.5	26.5	431
0330	24.2	1.5			10.0	61.3	539
0400	22.2	2.7			7.5	47.0	490
0430	31.4	4.8			11.0	33.8	382
0500	39.4	5.1			9.5	41.2	510
0530	38.4	4.5			8.0	27.9	402
0600	40.1	7.2			7.5	53.9	598
0630	NO	SAMPLE			-	-	-
0700	31.8	5.4			15.5	36.3	549
0730	30.4	9.8			17.0	36.0	564
0800	38.6	6.2			15.0	57.8	480
0830	39.6	3.1			13.0	37.7	539
0900	NO	SAMPLE			-	-	-
0930	41.4	4.7			13.0	34.8	578
1000	50.4	6.9			13.0	46.1	510
1030	53.3	9.5			11.0	32.3	382
1100	53.3	7.7			10.5	46.1	500
1130	46.7	6.6			10.5	34.8	289
1200	44.9	6.0			9.5	26.0	676
1230	42.5	9.2			13.0	25.5	598
1300	49.5	4.2			13.0	38.2	309

subject no. 9 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
1330	45.5	6.6	No Samples		9.0	31.4	735
1400	52.1	5.3			6.0	28.4	382
1430	40.1	5.2			7.0	39.2	549
1500	38.1	4.0			9.0	20.1	372
1530	42.8	2.0			6.0	39.2	559
1600	45.1	1.0			9.0	30.4	372
1630	52.8	5.6			13.0	42.1	461
1700	42.0	5.2			11.0	41.2	490
1730	44.3	6.0			11.0	36.3	470
1800	50.8	3.9			8.5	38.2	461
1830	32.9	1.0			8.0	51.5	828

Subject No. 10

Age: 62 Height: 173 cm Weight: 70 Kg % Ideal Weight: + 12%

Years since menopause: 8

Symptoms: Flushes (no intercourse)

Date of study: 23-24/10/76
Oestrogen therapy: Piperazine oestrone sulphate 3mg 1800h 23/10/76. Subsequent relief of flushes during the treatment cycle, but no withdrawal bleeding.

Hormone concentrations:

Time	E_1	E_2
1400	3.7	4.4
1430	1.1	3.4
1500	2.1	4.3
1530	1.0	3.7
1600	1.1	4.9
1630	1.5	5.1
1700	1.0	2.9
1730	"	3.8
1800	2.4	5.4
1830	2.3	4.7
1900	12.8	5.8
1930	15.3	5.3
2000	20.1	6.8
2030	14.1	5.7
2100	14.4	2.9
2130	13.9	3.7
2200	14.4	4.3
2230	17.4	3.8
2300	26.4	4.0
2330	18.4	1.7
2400	17.3	4.5
0030	22.0	4.4
0100	24.9	5.3
0130	23.4	5.3
0200	15.5	-
0230	17.3	5.9
0300	13.0	5.2
0330	16.9	6.5
0400	27.5	6.7
0430	18.0	7.0
0500	15.6	8.2
0530	25.0	8.3
0600	15.3	6.7
0630	16.2	7.7
0700	15.4	5.5
0730	20.1	6.8

subject no. 10 (cont.)

Time	E_1	E_2
0800	21.4	4.9
0830	27.6	4.6
0900	15.1	6.7
0930	25.3	6.8
1000	17.2	7.2
1030	18.8	7.6
1100	23.8	7.8
1130	20.6	7.8
1200	16.2	-
1230	27.3	-
1300	34.7	7.2
1330	28.0	6.3
1400	21.7	6.2

Subject No. 11

Age: 51 Height: 158 cm Weight: 51 Kg % Ideal Weight: -0.5%

Years since menopause: 2.6

Symptoms: Flushes (no intercourse)

Date of study: 20-21/11/76
Oestrogen treatment: Oestradiol valerate 1mg 1300h 20/11/76. Subsequent relief of flushes during the treatment cycle but no withdrawal bleeding.

Exact time of flushes: 1227; 1258; 1332; 1407; 1441; 1538; 1633; 1930; 1955; 2043; 2054; 2220; 0010; 0130; 0240; 0335; 0650; 0822; 0833 (hours)

Hormone concentrations:

Time	E_1	E_2
0900	3.3	2.1
0930	3.7	1.3
1000	2.8	1.6
1030	3.1	2.1
1100	2.5	2.0
1130	3.4	2.0
1200	2.6	1.6
1230	2.9	1.1
1300	1.9	3.1
1330	2.9	1.6
1400	4.8	2.6
1430	8.9	3.0
1500	9.1	4.9
1530	16.9	6.5
1600	14.5	6.0
1630	17.4	4.8
1700	13.3	3.8
1730	11.9	3.8
1800	19.1	-
1830	20.7	4.5
1900	16.7	4.1
1930	15.9	3.4
2000	18.6	4.5
2030	13.3	2.9
2100	18.3	3.7
2130	17.3	-
2200	13.6	5.0
2230	-	4.2
2300	13.0	3.8
2330	8.3	3.8
2400	12.1	3.5
0030	14.8	3.8

subject no. 11 (cont.)

Time	E_1	E_2
0100	13.2	5.6
0130	13.8	4.0
0200	11.2	3.8
0230	12.8	2.8
0300	9.1	2.4
0330	9.7	-
0400	7.7	2.5
0430	10.9	2.9
0500	14.3	2.5
0530	12.0	2.3
0600	8.5	2.8
0630	8.1	2.6
0700	7.7	2.2
0730	8.7	2.3
0800	7.7	2.1
0830	9.0	2.6

Subject No. 12

Age: 51 Height: 165 cm Weight: 57 Kg % Ideal Weight + 5.5%

Years since menopause: 0.9

Symptoms: Both flushes and superficial dyspareunia

Oestrogen therapy: Prescribed oestradiol valerate cyclically subsequent to study but no relief of flushes or withdrawal bleeding

Date of study: 28-29/11/76
Oestrogen administered: oestradiol valerate 1mg 1930h 28/11/76

Hormone concentrations:

Time	E_1	E_2
1530	5.9	4.0
1600	5.4	3.5
1630	5.7	3.9
1700	5.8	5.5
1730	5.6	4.3
1800	5.4	3.8
1830	5.3	1.9
1900	5.1	1.3
1930	4.6	1.7
2000	10.9	2.7
2030	12.4	2.6
2100	14.1	8.5
2130	16.5	4.7
2200	21.0	3.7
2230	17.8	5.3
2300	17.5	5.0
2330	12.7	5.0
2400	6.6	6.9
0030	7.8	5.5
0100	7.5	5.8
0130	9.1	6.1
0200	9.7	5.0
0230	9.0	5.2
0300	9.4	4.4
0330	8.4	3.9
0400	13.0	4.8
0430	15.1	4.5
0500	10.2	3.7
0530	13.6	3.6
0600	9.5	4.7
0630	10.4	3.9
0700	9.6	4.0
0730	10.8	3.9
0800	15.0	4.1

subject no. 12 (cont.)

Time	E_1	E_2
0830	15.5	5.3
0900	16.3	5.3
0930	12.8	6.2
1000	16.5	5.6
1030	14.7	6.7
1100	14.0	6.4
1130	7.3	5.9
1200	6.0	7.0
1230	8.7	4.6
1300	9.0	3.8
1330	8.7	1.7
1400	12.5	2.4
1430	7.9	2.4
1500	9.3	3.2
1530	8.4	3.0

Subject No. 13

Age: 54 Height: 167 cm Weight: 60 Kg % Ideal Weight: + 3.3%

Years since menopause: 3.0

Oestrogen therapy: Piperazine oestrone sulphate prescribed 0.5 years prior to study (first) for flushes and dyspareunia. Effective relief on 3mg/day cyclically In first study investigated on day 21 of the cycle - experiencing withdrawal bleeds.

Date of first study: 16-17/10/76
Oestrogen administered: Piperazine oestrone sulphate 3mg 1800h 16/10/76

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1400	94.8	26.3	25	46.7	20.4	-	180
1430	137.8	20.9	30	53.3	23.1	4.7	140
1500	113.3	18.9	17	37.3	21.4	5.0	130
1530	102.2	23.0	38	<26.6	16.7	4.7	110
1600	83.1	20.4	38	"	13.2	4.7	140
1630	119.1	18.0	32	"	12.2	5.0	130
1700	107.7	24.3	36	"	10.5	5.3	125
1730	79.9	23.9	35	"	8.4	-	110
1800	71.4	24.6	22	"	7.2	4.6	130
1830	93.8	19.1	29	"	8.4	3.2	120
1900	93.3	22.4	30	"	4.7	5.2	125
1930	125.7	-	24	"	6.0	4.6	129
2000	94.9	23.3	28	"	5.3	5.1	100
2030	94.8	19.6	19	"	3.2	4.2	145
2100	71.8	-	15	"	3.1	6.3	105
2130	-	-	17	"	2.7	3.7	113
2200	113.4	21.4	18	"	1.0	4.5	115
2230	116.9	16.4	12	"	2.1	3.3	105
2300	121.6	16.4	13	"	6.2	5.3	135
2330	95.2	16.3	15	"	5.6	4.7	140
2400	84.6	16.0	21	"	4.8	6.0	115
0030	92.7	19.4	17	30.3	8.6	4.9	123
0100	90.0	24.3	15	<26.6	5.4	5.0	110
0130	86.1	19.1	14	"	6.6	3.8	113
0200	104.1	21.1	20	"	6.5	4.7	123
0230	91.5	21.4	14	"	2.9	4.2	100
0300	122.5	23.9	16	"	4.3	4.7	85
0330	100.7	20.6	25	39.5	20.6	5.3	130
0400	91.4	26.2	20	54.7	25.9	2.7	125
0430	100.1	20.4	16	39.5	25.2	2.1	125
0500	102.4	23.6	20	45.3	22.7	3.7	130
0530	NO SAMPLE			-	-	-	-
0600	123.1	24.2	15	39.5	18.8	3.8	125
0630	75.8	24.4	15	41.3	18.8	4.5	125

subject no. 13 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0700	118.1	23.9	24	26.6	16.9	6.5	113
0730	101.9	25.3	25	64.0	24.7	6.8	125
0800	127.0	16.2	31	86.7	>30.0	5.4	88
0830	116.4	19.0	26	46.7	27.2	7.8	125
0900	90.7	19.4	16	<26.6	24.9	4.7	120
0930	107.3	18.6	21	"	20.9	6.3	105
1000	136.8	20.5	21	"	23.1	6.1	113
1030	84.4	18.6	18	"	18.1	4.9	105
1100	96.2	21.0	16	"	14.7	4.7	105
1130	103.6	21.8	18	"	11.1	4.2	105
1200	100.7	25.1	19	"	15.5	4.9	90
1230	84.2	21.2	19	30.7	19.1	3.3	108
1300	135.4	22.3	26	<26.6	20.7	5.0	108
1330	110.4	26.9	21	"	7.3	5.1	95
1400	123.6	24.1	25	"	15.3	5.3	103
1430							

Date of second study: 29-30/4/77 - prior to this study and after the first study, developed breakthrough bleeding and in January 1977 an endometrial aspirate showed cystic hyperplasia. Oestrogen therapy discontinued 24/1/77. Readmitted for second study 13 weeks later.

Symptoms: Flushes but no superficial dyspareunia

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1900	4.6	3.0	29	128.0	6.1	No Samples	
1930	4.2	3.7	22	120.6	2.8		
2000	2.4	2.8	20	46.7	3.4		
2030	4.4	2.8	20	42.6	2.8		
2100	4.1	2.5	24	26.6	1.5		
2130	3.7	2.1	22	77.3	2.1		
2200	3.7	3.5	31	114.7	3.2		
2230	5.9	2.8	30	101.3	2.7		
2300	4.4	3.8	35	90.7	3.8		
2330	6.0	2.3	31	46.7	1.3		
2400	6.3	3.8	30	<26.6	1.5		
0030	5.5	4.0	26	"	1.0		
0100	5.9	2.7	26	"	1.1		
0130	5.0	3.7	27	"	1.2		
0200	5.3	2.1	-	"	5.9		
0230	6.3	2.5	21	"	2.4		
0300	5.1	3.0	32	46.7	9.8		
0330	<1.0	2.7	24	<26.6	7.1		
0400	2.5	3.4	26	"	5.4		
0430	<1.0	2.6	19	"	4.2		
0500	2.5	2.6	20	49.3	13.9		
0530	3.5	3.3	21	98.7	17.6		
0600	4.5	3.0	25	74.7	18.8		
0630	5.3	2.6	25	49.3	14.5		
0700	4.0	4.5	24	73.3	14.7		
0730	1.2	3.0	22	<26.6	20.9		

subject no. 13 second study (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0800	3.1	2.5	19	56.0	12.1	No Samples	
0830	3.2	2.2	17	72.0	10.0		
0900	2.2	1.7	17	<26.6	7.3		
0930	4.5	<1.0	16	45.3	6.4		
1000	3.0	-	20	<26.6	7.0		
1030	3.6	2.8	27	36.0	12.5		
1100	1.5	2.6	21	36.0	13.0		
1130	3.4	3.4	26	<26.6	8.1		
1200	4.6	2.6	18	"	8.1		
1230	5.4	2.6	28	29.3	14.4		
1300	5.3	3.0	17	65.3	10.3		
1330	6.4	3.4	22	-	7.2		
1400	5.5	1.8	20	<26.6	6.6		
1430	4.9	3.1	25	"	5.3		
1500	5.1	-	23	-	6.1		
1530	<1.0	2.6	17	-	6.4		
1600	2.6	2.7	15	122.7	5.7		
1630	2.1	2.7	13	72.0	5.1		
1700	3.1	1.9	15	78.7	5.0		
1730	<1.0	1.8	16	65.3	5.1		
1800	4.3	3.0	22	58.7	10.1		

Subject No. 14

Age: 51 Height: 170 cm Weight: 58.5 % Ideal Weight - 9%

Years since menopause: 1.6

Symptoms: Both flushes and superficial dyspareunia

Date of study: 29-30/8/76
oestrogen treatment: Oestradiol valerate 2mg 1300h 29/8/76. Relief of flushes but no withdrawal bleeding during subsequent treatment cycle.

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0900	3.1	3.4	38	80.0	12.5	21.6	250
0930	2.7	2.2	35	69.3	13.5	20.6	245
1000	2.5	1.6	38	60.0	10.0	20.6	279
1030	3.4	4.1	33	44.0	9.0	25.5	230
1100	3.5	2.2	35	45.3	8.5	24.5	279
1130	3.1	1.7	42	58.7	10.0	19.1	250
1200	4.2	2.6	46	80.0	10.0	24.0	402
1230	5.3	<1.0	47	72.0	15.0	23.5	348
1300	5.3	"	42	46.7	14.0	21.1	319
1330	5.5	"	45	52.0	11.0	26.5	230
1400	18.6	2.7	-	45.3	-	24.0	319
1430	17.1	3.3	-	53.3	-	21.6	333
1500	17.6	3.2	35	52.0	6.5	23.5	250
1530	20.1	7.3	26	48.0	6.5	22.5	255
1600	21.5	4.6	37	46.7	5.5	20.6	333
1630	28.2	5.9	29	50.7	4.5	25.5	319
1700	23.5	6.5	18	49.3	4.5	28.9	397
1730	27.5	7.7	17	41.3	5.0	25.0	421
1800	25.6	6.7	18	41.3	4.5	27.0	304
1830	28.7	4.9	40	53.3	5.5	25.0	289
1900	25.2	5.3	26	45.3	6.0	25.5	372
1930	24.2	4.1	28	52.0	8.0	33.3	480
2000	23.4	3.6	47	34.7	4.5	33.3	304
2030	30.5	-	49	60.0	3.5	35.3	279
2100	30.2	3.8	59	45.3	3.0	19.6	235
2130	31.3	3.3	35	33.3	3.0	28.9	289
2200	28.5	2.1	42	<26.6	2.0	19.1	245
2230	31.3	2.6	42	36.0	2.5	25.0	279
2300	18.9	1.9	54	34.7	1.5	21.1	230
2330	18.3	3.5	52	32.0	1.5	19.6	274
2400	19.8	2.4	43	30.7	2.0	16.2	382
0030	18.6	1.9	43	30.7	1.5	16.2	333
0100	18.9	2.2	18	32.0	2.5	27.0	245
0130	18.9	-	20	33.3	3.5	28.9	289
0200	18.8	<1.0	18	41.3	5.0	29.9	353
0230	20.7	1.9	24	50.7	12.5	28.9	353

subject no. 14 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0300	17.6	2.0	29	42.7	7.5	19.6	353
0330	8.0	1.9	33	42.7	5.0	27.4	402
0400	8.7	1.0	24	37.3	4.5	25.5	333
0430	7.3	1.8	22	48.0	8.0	29.4	431
0500	10.9	1.2	28	64.0	12.0	25.5	421
0530	15.1	1.7	38	82.7	15.0	34.3	353
0600	9.4	3.2	29	54.7	13.0	28.9	245
0630	13.5	1.8	49	60.0	12.5	26.5	421
0700	12.9	2.1	58	69.3	11.5	30.4	353
0730	12.3	1.9	52	114.7	17.5	41.2	274
0800	14.0	<1.0	-	70.7	11.5	32.3	319
0830	11.9	2.7	-	88.0	16.5	34.3	235
0900	-	1.2	-	72.0	12.5	37.2	250

Subject No. 15

Age: 49 Height: 163 cm Weight: 56 Kg % Ideal Weight: + 1%

Years since menopause: 2.1

Oestrogen therapy: One year prior to study prescribed oestradiol valerate, for loss of libido, for 6 months. Wanted to have further trial of oestrogen therapy. No flushes or superficial dyspareunia - no subsequent withdrawal bleeding.

Date of study: 3-4/3/77
Oestrogen administered: Oestradiol valerate 1mg 2300h 3/3/77

Hormone concentrations:

Time	E_1	E_2
1900	6.1	1.1
1930	5.8	<1.0
2000	4.3	"
2030	5.5	5.2
2100	5.5	3.9
2130	6.2	3.4
2200	5.3	5.5
2230	5.5	3.1
2300	4.9	2.1
2330	6.7	2.4
2400	6.2	3.1
0030	10.4	5.0
0100	8.7	6.7
0130	7.6	3.9
0200	9.5	4.0
0230	7.9	3.3
0300	6.6	4.0
0330	8.7	2.4
0400	9.1	2.5
0430	8.4	3.3
0500	9.3	2.8
0530	10.1	3.0
0600	8.0	3.6
0630	9.3	2.8
0700	9.0	6.3
0730	8.2	3.9
0800	9.2	4.9
0830	7.7	3.3
0900	5.6	4.2
0930	7.2	3.4
1000	8.4	4.9
1030	7.8	2.9
1100	7.2	2.5
1130	4.5	3.2

subject no. 15 (cont.)

Time	E_1	E_2
1200	5.5	2.0
1230	7.9	1.8
1300	6.0	2.5
1330	6.5	4.6
1400	5.2	-
1430	6.8	2.4
1500	6.9	5.0
1530	6.5	1.9
1600	8.9	2.9
1630	8.4	1.9
1700	6.6	2.9
1730	8.4	2.4
1800	6.8	1.0
1830	7.3	1.8
1900	6.2	2.9

Subject No. 16

Age: 49 Height: 177 cm Weight: 71 Kg % Ideal Weight: + 9%

Years since menopause: 0.9

Symptoms: Neither flushes nor superficial dyspareunia

Date of study: 3-5/7/76
Dexamethasone treatment: 2mg 2400h 4/7/76

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2200	5.7	6.8	-	77.3	2.5	51.5	554
2230	6.5	6.1	25	98.7	3.0	59.8	480
2300	4.1	5.2	28	88.0	3.0	70.0	363
2330	8.1	4.9	32	106.7	4.0	61.3	446
2400	4.7	4.6	30	66.7	2.5	52.9	412
0030	7.5	4.3	30	98.7	3.5	34.8	402
0100	11.2	4.9	30	64.0	3.5	44.6	505
0130	9.6	5.5	25	93.3	5.0	41.7	554
0200	11.9	6.2	23	74.7	5.0	34.8	353
0230	9.4	5.9	23	80.0	4.5	35.3	304
0300	9.1	5.1	23	80.0	2.5	39.2	451
0330	3.8	4.7	23	88.0	4.0	39.7	402
0400	3.6	6.3	22	160.0	13.0	45.1	304
0430	3.8	5.5	22	106.7	9.5	38.2	554
0500	4.0	5.2	22	88.0	9.0	40.7	652
0530	4.5	4.6	25	80.0	7.0	41.2	470
0600	2.9	5.4	28	80.0	5.5	41.2	500
0630	3.0	6.8	22	138.7	6.5	42.6	353
0700	4.2	5.1	23	149.3	9.5	39.7	480
0730	4.5	-	23	133.3	9.5	43.1	481
0800	2.4	5.8	25	149.3	10.5	43.6	529
0830	5.1	5.0	22	109.3	10.0	36.3	353
0900	3.0	5.5	-	78.7	8.5	31.4	554
0930	3.7	4.2	25	90.7	8.0	35.3	304
1000	2.1	3.8	25	64.0	6.0	39.2	363
1030	2.2	5.1	28	78.7	5.5	38.2	431
1100	5.4	5.0	28	78.7	-	41.7	510
1130	4.8	5.1	32	56.0	3.0	37.7	412
1200	6.2	4.0	32	77.3	3.5	33.3	319
2200	5.6	4.5	28	62.7	<2.5	32.3	686
2230	5.5	6.3	25	68.0	"	32.3	637
2300	6.2	5.0	28	96.0	3.0	37.7	735
2330	7.9	8.0	32	144.0	10.0	44.6	441
2400	8.5	8.1	30	77.3	8.0	36.3	353
0030	11.4	5.9	30	72.0	6.5	39.2	441
0100	11.2	5.9	30	77.3	6.0	37.2	431

subject no. 16 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0130	8.7	5.3	25	45.3	5.0	37.2	363
0200	7.7	6.4	23	48.0	4.5	34.3	304
0230	10.0	6.7	23	53.3	4.0	40.2	441
0300	8.2	5.5	23	53.3	3.5	51.5	412
0330	8.1	5.9	23	44.0	2.5	42.1	402
0400	1.0	4.9	22	45.3	2.5	44.6	353
0430	1.8	5.4	22	32.0	2.5	43.6	402
0500	<1.0	4.4	22	26.0	4.0	47.0	588
0530	"	5.7	25	33.3	1.5	43.6	441
0600	"	4.3	28	26.7	4.5	53.9	470
0630	1.3	6.6	22	32.0	<1.0	34.3	470
0700	2.9	4.3	23	34.7	2.0	37.2	451
0730	3.1	7.1	23	32.0	1.5	42.1	500
0800	2.5	5.2	25	26.7	1.5	35.3	519
0830	2.3	5.4	22	30.7	-	27.4	353
0900	N O	S A M P L E		-	-	-	-
0930	1.4	6.1	25	33.3	1.5	30.4	431
1000	2.1	6.0	25	33.3	1.5	35.8	515
1030	2.9	6.5	28	34.7	1.0	23.5	652
1100	4.0	6.3	28	58.7	1.0	35.8	510
1130	3.6	-	32	63.3	12.0	35.8	554
1200	2.3	-	-	72.0	-	36.3	470

Subject No. 17

Age: 46 years Height: 170 cm Weight: 67Kg % Ideal Weight: + 4.6%

Years since ovariectomy: 0.4

Indication for ovariectomy: Endometriosis

Symptoms: Flushes but no superficial dyspareunia

Date of study: 24-25/1/76

Exact time of flushes: 0915; 0959; 1115; 1124; 1204; 1230; 1233; 1352; 1457; 1741; 2032; 0505 (hours)

Times asleep: 2320-0320; 0520-0600.

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0800	6.7	5.3	32	114.7	7.0	36.8	490
0820	8.2	3.0	23	85.3	5.5	29.4	431
0840	11.4	4.9	40	93.3	2.5	25.0	461
0900	14.1	3.8	32	88.0	4.5	31.4	372
0920	12.5	6.8	38	106.6	8.5	28.9	323
0940	9.5	2.7	31	88.0	3.0	23.0	470
1000	11.3	5.7	29	80.0	2.0	24.0	421
1020	8.9	5.9	37	128.0	4.0	29.9	431
1040	9.5	3.3	33	109.3	6.5	31.4	387
1100	9.3	5.0	33	93.3	4.0	27.4	402
1120	6.7	5.0	32	66.7	1.5	30.4	421
1140	8.8	2.8	31	133.3	4.0	24.5	461
1200	7.9	4.8	28	104.0	7.0	28.9	431
1220	8.4	4.8	32	93.3	4.0	18.6	431
1240	8.7	3.9	32	149.3	7.5	25.0	470
1300	8.0	3.2	32	120.0	7.0	25.0	451
1320	4.2	4.8	27	80.0	11.5	24.5	461
1340	4.4	4.3	31	61.3	4.0	21.6	451
1400	5.3	5.2	23	59.7	4.0	27.4	613
1420	5.5	1.8	27	77.3	4.5	27.4	510
1440	11.3	6.0	31	84.0	5.5	41.2	686
1500	10.4	5.2	27	69.3	2.5	36.3	956
1520	5.6	3.2	31	45.3	8.5	53.9	907
1540	6.3	4.6	33	77.3	6.0	48.0	774
1600	8.2	4.5	31	69.3	2.5	33.3	735
1620	6.9	5.0	32	98.7	1.5	31.9	872
1640	7.8	6.2	33	154.7	4.0	25.0	784
1700	6.9	3.8	30	178.0	3.5	26.5	892
1720	10.6	3.2	32	170.7	4.0	29.4	750
1740	5.9	4.6	35	173.3	3.5	26.5	431
1800	4.2	4.7	22	50.0	2.5	24.5	529
1820	8.2	4.0	27	70.7	3.0	28.9	461
1840	1.8	6.1	28	109.3	12.5	28.9	451

subject no. 17 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
1900	3.5	4.3	28	104.0	9.0	28.4	461
1920	2.0	3.7	18	72.0	9.5	28.9	421
1940	2.1	1.9	32	53.3	4.0	23.5	421
2000	2.2	4.7	19	57.3	2.0	26.5	402
2020	1.0	8.2	19	50.7	4.5	28.9	348
2040	8.2	7.6	18	40.0	3.0	24.5	490
2100	7.9	3.9	27	42.7	2.0	28.9	402
2120	7.4	4.4	32	32.0	2.0	24.5	461
2140	4.0	2.1	29	58.7	2.5	30.4	549
2200	3.9	3.0	33	64.0	3.5	33.8	431
2220	5.2	2.9	33	77.3	3.0	33.3	480
2240	4.2	4.3	38	72.0	4.0	33.8	372
2300	2.2	3.1	41	53.3	2.0	35.3	490
2320	5.9	3.8	38	45.3	2.0	26.5	392
2340	4.0	2.8	36	40.0	1.0	41.2	431
2400	3.4	2.6	33	37.3	1.0	27.9	539
0020	2.4	4.0	32	-	-	-	-
0040	2.0	2.2	36	33.3	2.0	23.0	715
0100	1.8	4.2	38	26.6	1.0	26.0	578
0120	<1.0	3.9	42	62.7	4.5	29.4	500
0140	<1.0	2.5	42	45.3	2.0	27.4	613
0200	<1.0	4.3	43	40.0	2.5	26.5	431
0220	2.0	2.7	42	42.7	2.0	25.0	319
0240	2.3	3.1	47	133.3	7.5	28.4	402
0300	6.2	2.4	42	96.0	6.5	25.0	431
0320	3.7	3.0	46	-	11.0	28.9	387
0340	3.4	1.8	42	64.0	10.0	25.0	402
0400	6.4	1.5	36	78.7	2.0	24.0	319
0420	4.6	3.1	32	77.3	5.0	24.5	402
0440	8.8	2.7	38	88.0	11.0	30.4	372
0500	3.6	2.3	32	112.0	6.5	27.9	382
0520	2.0	2.3	32	105.3	2.0	26.5	397
0540	4.5	2.9	41	186.7	15.0	38.2	470
0600	1.5	3.1	41	256.0	9.0	38.2	421
0620	2.5	2.3	-	-	-	-	-
0640	5.5	4.7	36	280.0	11.0	-	-
0700	3.4	5.3	36	202.7	13.5	29.4	647
0720	NO	SAMPLE		-	-	-	-
0740	4.6	7.2	42	192.0	13.5	27.9	480
0800	<1.0	5.7	34	133.3	-	26.0	725

Subject No. 18

Age: 49 Height: 170 cm Weight: 65 Kg % Ideal Weight + 1.5%

Years since ovariectomy: 14

Indication for ovariectomy: Fibroids, with ovarian fibroma

Symptoms: Flushes but no superficial dyspareunia

Date of study: 17-18/7/76

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1000	6.5	5.2	25	58.5	8.0	21.6	245
1020	7.4	6.4	20	52.0	7.0	25.0	402
1040	6.6	7.2	23	50.7	7.0	26.5	402
1100	6.7	3.6	23	45.3	6.0	24.5	480
1120	6.1	4.4	19	52.0	5.0	30.4	294
1140	4.4	4.0	25	50.7	5.0	27.9	382
1200	6.0	5.0	16	45.3	5.0	29.4	270
1220	4.5	4.9	19	40.0	5.0	27.9	323
1240	3.8	4.2	12	45.3	5.0	31.4	353
1300	6.0	4.8	11	34.7	4.0	28.4	451
1320	6.0	4.5	17	45.3	5.0	35.3	412
1340	4.9	3.9	16	61.3	5.0	36.3	441
1400	4.8	4.3	16	36.0	5.0	34.3	392
1420	4.3	4.0	10	36.0	4.0	20.6	431
1440	3.9	5.4	16	36.0	3.0	23.0	431
1500	3.8	2.9	19	34.7	3.0	24.5	441
1520	4.2	3.6	12	50.7	4.0	33.3	441
1540	4.5	5.1	23	61.3	8.0	25.5	402
1600	5.5	3.8	12	45.3	5.0	25.0	431
1620	4.8	3.5	16	37.3	4.0	22.1	515
1640	5.0	5.1	25	32.0	4.0	21.1	480
1700	4.3	5.3	19	32.0	4.0	25.5	480
1720	4.1	5.8	16	26.7	4.0	22.5	294
1740	4.7	6.5	17	41.3	4.0	20.6	304
1800	2.8	6.0	12	32.0	3.0	23.0	510
1820	3.9	6.6	17	30.7	4.0	27.9	353
1840	4.3	6.0	16	32.0	4.0	24.0	402
1900	5.8	4.6	17	38.7	4.0	25.5	363
1920	4.7	5.8	16	37.3	3.0	24.5	402
1940	4.3	4.9	17	42.7	4.0	30.4	402
2000	3.6	5.9	17	32.0	3.0	30.4	441
2020	4.8	5.0	11	28.0	3.0	33.3	441
2040	6.0	5.2	16	38.7	2.0	26.5	279
2100	5.1	7.8	10	26.6	2.0	29.4	319
2120	5.2	4.7	11	32.0	2.0	24.5	162
2140	5.8	3.5	11	41.3	2.0	31.9	304
2200	4.3	3.9	12	26.6	3.0	28.9	323
2220	3.3	4.2	19	29.3	3.0	27.9	421

subject no. 18 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2240	3.9	3.9	16	26.6	3.0	29.9	412
2300	3.6	4.7	17	28.0	4.0	27.4	323
2320	4.5	3.9	10	26.6	3.0	28.9	319
2340	5.9	4.5	17	30.7	3.0	26.0	402
2400	3.9	4.8	19	33.3	3.0	24.5	363
0020	5.5	5.1	11	32.0	1.0	31.4	294
0040	4.1	4.9	12	42.7	3.0	28.4	470
0100	2.3	4.1	23	40.0	3.0	31.4	451
0120	6.3	5.9	17	36.0	3.0	29.9	480
0140	5.7	6.0	23	80.0	5.0	34.8	250
0200	4.4	5.3	30	62.7	1.0	34.3	421
0220	4.3	6.6	19	50.7	6.0	30.9	421
0240	3.6	7.7	16	49.3	5.0	36.3	568
0300	4.6	3.8	16	53.3	5.0	36.3	461
0320	4.1	4.8	11	52.0	12.0	29.4	441
0340	3.9	4.8	11	58.7	11.0	31.9	627
0400	3.1	6.3	12	56.0	8.5	28.9	539
0420	2.9	3.6	10	50.7	8.5	22.1	554
0440	4.5	3.4	12	90.7	12.5	30.4	617
0500	3.1	4.2	23	96.0	16.5	27.4	421
0520	3.2	4.1	37	85.3	18.0	34.3	353
0540	5.0	3.8	19	93.3	17.0	31.9	539
0600	5.3	4.1	30	74.7	17.5	33.3	245
0620	5.8	3.9	30	66.7	14.5	36.3	279
0640	5.6	4.8	20	69.3	13.5	36.3	363
0700	4.1	4.6	28	68.0	15.0	21.1	382
0720	4.4	5.0	27	77.3	15.0	31.4	245
0740	NO SAMPLE			-	-	-	-
0800	4.4	6.0	27	64.0	-	32.8	412
0820	4.0	5.0	28	72.0	11.5	30.4	441
0840	2.8	5.3	28	73.3	14.5	31.4	451
0900	4.4	5.7	30	73.3	9.0	33.3	515
0920	3.3	6.2	16	53.3	11.0	34.3	431
0940	4.5	5.5	19	-	10.5	30.4	441
1000	4.1	6.0	23	62.7	9.5	31.4	510

Subject No. 19

Age: 50 years Height: 173 cm Weight: 63 Kg % Ideal Weight: + 2.4%

Years since ovariectomy: 3

Indication for ovariectomy: Menorrhagia treated by hysterectomy

Symptoms: Flushes but no superficial dyspareunia

Date of first study: 20-21/1/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	6.0	3.8	64	26.6	1.8	No Samples	
2430	6.0	3.2	64	106.7	2.5		
0100	8.2	2.6	59	81.3	2.5		
0130	5.4	2.2	72	48.0	5.9		
0200	4.7	2.4	69	82.7	13.1		
0230	6.2	2.4	86	110.7	17.1		
0300	5.6	2.4	84	110.7	20.3		
0330	3.9	1.6	80	118.7	23.0		
0400	4.4	3.2	59	116.0	21.2		
0430	3.6	2.8	49	93.3	16.6		
0500	4.3	3.5	49	89.3	14.0		
0530	5.0	4.2	44	96.0	10.5		
0600	3.8	4.4	39	93.3	13.4		
0630	4.6	3.8	30	92.0	11.2		
0700	5.4	3.3	36	54.7	13.8		
0730	5.3	2.2	33	96.0	24.0		
0800	4.8	3.5	44	128.0	22.8		
0830	4.7	2.3	40	105.3	22.0		
0900	4.0	2.8	47	74.0	19.4		
0930	NO SAMPLE			-	-		
1000	3.7	<1.0	<10	80.0	12.6		
1030	3.7	1.4	42	70.7	14.3		
1100	4.4	1.6	55	73.3	8.1		
1130	4.4	1.3	47	77.3	9.2		
1200	5.0	2.3	35	52.6	5.6		

Date of second study: 27-28/1/77

Dexamethasone treatment: Prescribed on discharge after first study - 0.5 mg t.d.s. (0800, 1700, 2400)
Tetracosactrin infusion: 0830-1430 28/1/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	<1.0	<1.0	33	<26.6	<1.0	No Samples	
0030	"	"	19	"	"		

subject no. 19 second study (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0100	<1.0	1.4	10	<26.6	<1.0	No Samples	
0130	"	1.9	15	"	"		
0200	"	<1.0	23	"	"		
0230	"	1.3	17	"	"		
0300	1.3	2.0	19	28.0	"		
0330	1.2	2.6	17	<26.6	"		
0400	<1.0	<1.0	25	30.0	"		
0430	"	1.8	26	<26.6	"		
0500	"	1.5	<10	"	"		
0530	"	<1.0	"	"	"		
0600	"	"	18	"	"		
0630	"	"	18	"	"		
0700	1.2	"	10	"	"		
0730	1.0	"	18	"	"		
0800	<1.0	1.2	24	"	1.2		
0830	"	1.5	15	"	<1.0		
0900	"	<1.0	11	43.0	6.1		
0930	1.6	1.2	18	34.7	13.3		
1000	3.1	2.0	32	37.3	11.5		
1030	3.0	1.5	21	65.3	14.2		
1100	2.8	2.0	21	48.0	15.1		
1130	3.4	1.8	28	53.3	17.8		
1200	3.0	2.1	15	48.5	17.7		
1230	4.6	2.3	32	65.3	21.5		
1300	4.0	2.4	31	45.9	19.0		
1330	3.9	1.5	35	43.0	20.9		
1400	2.7	2.4	17	44.7	18.5		
1430	2.6	4.0	17	48.5	21.3		
1500	3.2	<1.0	15	54.7	19.1		
1530	3.8	2.0	30	43.0	17.1		
1600	3.6	<1.0	21	28.0	13.8		
1630	3.2	<1.0	19	32.0	10.3		
1700	3.9	1.4	21	<26.6	7.9		
1730	4.2	1.4	15	"	7.3		
1800	4.3	1.2	25	"	5.2		
1830	4.7	<1.0	25	"	4.0		

Subject No. 20

Age: 42 years Height: 175 cm Weight: 72 Kg % Ideal Weight: + 14%

Years since ovariectomy: 4

Indication for ovariectomy: Endometriosis

Symptoms: Flushes and superficial dyspareunia

Date of study: 12-14/1/77
Dexamethasone treatment: 2 mg 2400h 13/1/77,
0.5 mg 0800h & 1600h 14/1/77
Tetracosactrin infusion: 0830-1430h 14/1/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2300	3.3	2.5	31	50.7	4.5	No Samples	
2330	5.5	2.8	34	69.3	2.5		
2400	3.3	3.1	31	45.3	3.2		
0030	4.4	2.5	36	29.3	2.8		
0100	5.6	-	33	53.3	1.4		
0130	4.1	2.0	39	26.6	<1.0		
0200	5.0	2.7	32	37.3	"		
0230	5.6	1.6	39	48.0	3.8		
0300	3.3	1.7	29	57.3	3.7		
0330	2.3	2.2	22	109.3	7.3		
0400	3.3	2.4	21	56.0	4.6		
0430	2.0	2.6	27	62.7	3.9		
0500	2.5	1.9	26	88.0	3.9		
0530	2.3	2.4	21	58.7	6.1		
0600	3.1	2.1	32	45.3	5.9		
0630	2.9	2.3	39	130.7	12.6		
0700	2.2	2.3	45	154.7	16.0		
0730	2.3	2.8	32	152.0	23.1		
0800	2.8	1.9	29	133.3	22.9		
0830	3.0	2.5	34	98.7	17.4		
0900	2.3	2.2	21	64.0	16.2		
0930	2.1	2.0	21	73.3	10.3		
1000	2.1	2.4	21	106.7	11.6		
1030	3.1	2.5	32	85.3	10.1		
1100	3.1	2.0	34	101.3	8.7		
2300	2.1	2.3	22	33.3	1.1		
2330	3.4	1.4	21	36.0	1.0		
2400	2.2	2.2	26	34.7	2.2		
0030	2.2	2.5	23	32.0	1.0		
0100	2.5	2.3	22	50.7	1.0		
0130	2.2	1.8	24	34.7	2.9		
0200	1.7	2.1	19	32.0	2.2		
0230	2.3	1.6	38	28.0	1.2		

subject no. 20 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0300	<1.0	1.8	18	<26.6	1.0	No Samples	
0330	"	1.5	25	"	<1.0		
0400	"	1.3	16	"	1.0		
0430	"	1.5	16	"	1.7		
0500	"	1.1	13	"	1.1		
0530	"	1.9	NO SAMPLE -				
0600	"	1.9	<10	32.0	<1.0		
0630	"	1.9	"	33.3	"		
0700	"	1.7	"	<26.6	"		
0730	"	2.0	13	"	1.1		
0800	"	2.1	<10	"	<1.0		
0830	"	1.8	"	"	"		
0900	"	1.6	18	48.0	14.9		
0930	1.4	2.0	16	125.3	27.2		
1000	1.3	3.0	16	138.7	29.7		
1030	2.4	2.6	21	152.0	29.3		
1100	2.6	3.6	30	213.3	29.5		
1130	2.6	3.8	26	-	-		
1200	1.2	2.5	27	178.7	46.0		
1230	1.6	2.5	27	173.3	48.4		
1300	1.9	2.5	-	154.7	46.2		
1330	2.6	2.7	-	192.0	48.6		
1400	2.7	2.8	36	200.0	46.8		
1430	3.6	3.1	30	202.6	44.8		
1500	3.4	3.3	28	229.3	50.2		
1530	4.1	2.6	30	173.3	35.4		
1600	4.4	2.8	28	128.0	28.7		
1630	7.2	3.1	39	57.3	27.7		
1700	3.7	3.4	24	90.7	26.9		
1730	2.4	2.4	19	62.7	19.5		
1800	2.5	2.5	21	53.3	15.5		
1830	3.9	3.3	22	48.0	12.7		

Subject No. 21

Age: 47 Height: 163 cm Weight: 61 Kg % Ideal Weight: + 11%

Years since ovariectomy: 1.1

Indication for ovariectomy: Carcinoma of the cervix

Symptoms: Neither flushes nor superficial dyspareunia

Date of first study: 1-2/2/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	8.6	3.4	32	38.7	4.2	No Samples	
0030	7.5	2.1	-	26.6	1.8		
0100	9.0	2.9	-	<26.6	1.9		
0130	6.6	3.4	20	"	<1.0		
0200	9.4	2.6	23	33.3	"		
0230	8.6	2.0	24	26.6	"		
0300	8.2	2.6	24	32.0	"		
0330	8.3	1.9	22	<26.6	1.1		
0400	7.5	2.4	20	"	<1.0		
0430	7.2	2.9	26	42.7	5.1		
0500	8.3	2.6	31	45.3	7.6		
0530	9.0	3.5	31	106.7	13.8		
0600	8.9	3.3	33	117.3	14.1		
0630	9.1	3.7	30	122.7	10.5		
0700	8.3	3.4	28	100.0	7.1		
0730	8.4	3.8	31	93.3	14.2		
0800	6.9	3.2	24	96.0	12.7		
0830	7.8	2.8	25	74.7	9.9		
0900	7.7	4.6	26	53.3	3.8		
0930	8.0	3.6	26	41.3	5.1		
1000	8.7	3.7	30	30.7	3.5		
1030	8.6	5.4	26	89.3	6.3		
1100	7.5	3.4	30	34.7	3.2		
1130	9.2	1.7	32	69.3	5.8		
1200	8.6	2.9	35	106.7	12.1		

Date of second study: 8-9/2/77
Dexamethasone treatment: 0.5mg t.d.s. (0800, 1800, 2400h) prescribed for one week on discharge from first study and continuing through second
Androstenedione infusion: 0830-1430h 9/2/77

Hormone concentrations:

See Over:-

subject no. 21 second study (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2400	1.7	1.2	<10	<26.6	<1.0	No Samples	
0030	1.0	1.9	"	"	"		
0100	<1.0	1.8	"	"	"		
0130	"	1.8	"	"	"		
0200	"	1.2	"	"	"		
0230	"	1.2	"	"	"		
0300	"	1.2	"	"	"		
0330	"	1.9	"	"	"		
0400	"	1.1	"	"	"		
0430	2.0	1.7	"	"	"		
0500	3.7	1.7	"	"	"		
0530	3.5	<1.0	"	"	"		
0600	2.6	1.7	"	"	"		
0630	1.0	1.8	"	"	"		
0700	1.4	1.7	"	"	"		
0730	1.8	<1.0	"	"	"		
0800	1.2	"	"	"	"		
0830	<1.0	"	"	"	"		
0900	2.1	"	11	141.3	"		
0930	5.4	"	17	133.3	"		
1000	7.8	"	21	197.3	"		
1030	7.5	"	26	176.0	"		
1100	10.4	"	37	224.0	"		
1130	8.6	"	37	261.3	"		
1200	8.6	"	49	277.3	"		
1230	8.3	"	40	309.3	"		
1300	6.0	"	40	186.7	"		
1330	6.4	"	45	192.0	"		
1400	7.5	"	43	200.0	"		
1430	9.9	"	39	213.3	"		
1500	13.5	"	36	106.7	"		
1530	9.5	"	30	66.7	"		
1600	4.5	"	33	70.7	"		
1630	5.7	"	30	54.7	"		
1700	5.8	"	30	73.3	"		
1730	7.0	"	27	33.3	-		
1800	5.0	"	28	<26.6	-		
1830	5.5	"	25	"	-		

Subject No. 22

Age: 46 Height: 170 cm Weight: 65 Kg % Ideal Weight: 0%

Years since ovariectomy: 0.4

Indication for ovariectomy: Adenomyosis with an ovarian fibroma

Symptoms: Flushes (no intercourse)

Date of study: 12-13/4/77
Androstenedione infusion: 1600-2200h 12/4/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0400	5.9	2.9	30	26.6	7.0	9.0	500
0430	4.9	2.4	35	56.0	10.0	10.0	575
0500	5.6	3.3	35	66.7	10.1	9.3	575
0530	5.3	3.4	33	106.7	-	10.8	650
0600	1.0	3.0	39	113.3	17.2	10.0	600
0630	"	4.5	30	85.3	17.9	8.8	575
0700	"	3.4	33	80.0	19.8	7.0	550
0730	1.1	3.8	37	100.0	20.9	8.0	610
0800	1.0	2.8	35	82.7	24.2	10.0	625
0830	3.6	3.0	29	101.3	17.2	10.0	650
0900	1.9	2.6	29	26.6	16.0	8.8	575
0930	2.7	1.8	26	93.3	10.3	9.3	600
1000	1.0	3.3	27	82.7	-	9.3	575
1030	4.5	2.8	33	99.0	4.7	8.8	595
1100	4.3	2.4	34	113.3	8.1	9.5	625
1130	5.6	3.5	31	125.3	-	8.0	500
1200	1.0	2.5	43	157.3	18.4	-	625
1230	7.5	2.4	49	130.7	18.8	7.5	615
1300	5.0	3.5	34	105.3	12.1	8.5	540
1330	1.0	2.5	31	97.3	9.0	9.0	550
1400	"	3.7	29	100.0	-	8.3	550
1430	"	2.5	32	102.7	-	9.0	640
1500	"	3.3	26	101.3	10.8	8.8	475
1530	3.1	1.6	27	84.0	8.8	8.0	625
1600	3.7	2.4	35	96.0	13.6	9.0	710
1630	6.8	2.6	36	154.7	16.1	10.8	700
1700	7.8	2.5	41	120.0	17.0	8.8	650
1730	5.6	1.8	53	120.0	13.2	8.3	650
1800	5.5	3.4	46	133.3	8.2	7.5	655
1830	2.9	3.0	42	106.7	5.8	7.0	690
1900	7.2	3.4	50	224.0	8.7	8.0	510
1930	5.8	1.7	47	208.0	11.1	8.8	695
2000	5.0	2.9	52	123.3	8.9	7.7	575
2030	5.0	2.3	62	208.0	9.2	7.8	550
2100	5.2	2.5	53	186.7	6.4	9.0	535
2130	4.0	3.6	53	154.7	6.3	7.8	560
2200	4.9	3.1	53	160.0	2.8	7.1	565
2230	6.4	3.7	66	138.7	2.5	10.0	650
2300	6.8	3.8	68	82.7	5.0	9.0	625

subject no. 22 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2330	4.8	3.2	67	64.0	3.7	6.7	450
2400	6.1	2.5	53	78.7	5.7	12.5	565
0030	3.5	3.6	58	82.7	2.0	11.0	-
0100	8.6	2.9	51	53.3	2.5	8.3	560
0130	6.1	2.9	66	88.0	8.2	11.0	565
0200	5.5	3.9	66	132.0	17.5	13.0	575
0230	6.1	3.0	56	101.3	18.5	10.0	690
0300	6.5	-	59	112.0	13.0	9.8	600
0330	7.3	3.1	64	128.0	16.6	8.8	600
0400	5.0	3.0	64	101.3	12.9	9.5	575

Subject No. 23

Age: 54 Height: 168 cm Weight: 72 Kg % Ideal Weight + 24%

Years since ovariectomy: 5

Indication for ovariectomy: Carcinoma of the cervix

Symptoms: Neither flushes nor superficial dyspareunia

Date of study: 27/5/77

Androstenedione infusion: 1200-1800h 27/5/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0600	2.3	3.2	39	133.1	16.6	7.6	485
0630	1.0	2.7	40	114.7	19.0	7.6	450
0700	2.4	2.8	31	86.0	15.7	9.3	415
0730	1.4	4.1	23	73.3	25.5	7.6	415
0800	2.1	3.4	24	56.0	12.5	-	385
0830	2.5	3.1	25	68.8	10.3	8.3	455
0900	2.9	2.9	21	65.3	8.7	23.5	420
0930	2.7	3.3	28	89.3	13.8	15.5	375
1000	4.5	3.2	32	82.7	14.6	10.8	450
1030	5.3	3.0	34	42.7	10.8	9.2	420
1100	2.2	5.9	33	81.3	9.6	10.0	475
1130	4.9	2.5	34	62.7	6.0	10.3	450
1200	4.9	3.8	42	63.3	4.3	9.3	340
1230	4.7	3.5	43	160.0	8.9	7.8	495
1300	4.3	3.7	31	120.0	5.2	9.3	385
1330	6.3	3.5	36	124.0	7.0	7.1	425
1400	6.7	2.8	36	144.0	5.2	9.0	425
1430	6.8	3.9	40	202.7	11.2	8.0	415
1500	6.7	2.6	41	200.0	6.2	7.3	465
1530	6.0	3.3	49	242.7	-	8.5	445
1600	6.5	3.3	66	261.3	5.8	8.0	485
1630	9.4	3.6	54	272.0	3.6	10.0	495
1700	7.3	3.2	50	218.7	5.4	9.5	490
1730	9.2	3.5	66	242.7	-	9.8	455
1800	7.4	4.3	96	288.0	4.9	11.3	420
1830	6.6	4.0	70	149.3	8.4	9.5	510
1900	1.4	2.7	53	137.3	6.6	10.0	495
1930	1.0	2.2	-	106.7	5.9	9.0	465
2000	2.6	3.7	42	73.3	3.6	10.5	415
2030	4.5	3.8	44	72.0	4.3	9.3	425
2100	3.4	3.8	37	114.7	2.3	7.0	495
2130	4.3	3.8	43	52.7	2.5	8.8	405
2200	4.9	4.0	50	96.0	-	8.5	480
2230	6.1	3.3	57	70.7	4.0	9.8	440
2300	5.0	2.6	57	108.0	2.9	-	485
2330	6.8	4.1	45	40.5	2.2	-	425
2400	3.8	2.9	49	41.3	2.2	-	525

Subject No. 24

Age: 49 Height: 154 cm Weight: 60 Kg % Ideal Weight: + 20%

Years since ovariectomy: 6

Indication for ovariectomy: Endometriosis

Symptoms: Neither flushes nor superficial dyspareunia although oestrogen therapy trial indicated because of depression and anxiety

Date of study: 17-18/7/76
Oestrogen therapy: Piperazine oestrone sulphate 1.5mg 1410h

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1000	6.0	4.0	47	No Samples		32.3	647
1030	6.9	3.1	51			32.3	602
1100	6.7	4.0	44			41.2	578
1130	8.3	3.4	44			37.2	451
1200	9.8	3.9	34			24.5	372
1230	7.0	3.6	36			25.0	372
1300	7.9	1.5	34			21.6	421
1330	7.5	5.3	36			23.5	461
1400	6.6	3.3	44			28.4	431
1430	7.0	2.3	38			27.4	421
1500	9.6	2.9	38			25.0	392
1530	14.1	3.1	30			26.5	294
1600	12.5	3.2	46			20.1	402
1630	13.3	3.9	34			22.1	402
1700	16.9	3.8	38			23.0	461
1730	25.9	5.6	36			26.5	598
1800	19.3	4.8	34			23.0	564
1830	26.6	6.0	28			25.0	348
1900	28.9	5.3	40			29.4	470
1930	25.2	5.9	37			26.5	387
2000	22.7	6.4	52			37.2	353
2030	22.4	5.5	40			25.0	470
2100	23.0	-	33			42.1	500
2130	26.6	6.7	32			29.4	402
2200	24.9	7.0	36			28.4	735
2230	29.9	6.1	36			46.1	578
2300	27.4	5.9	38			34.8	353
2330	22.4	7.0	44			44.1	470
2400	23.7	6.2	44			34.8	402
0030	22.1	5.1	34			44.1	564
0100	21.3	5.9	44			26.5	431
0130	24.2	6.0	40			19.1	421
0200	29.7	6.2	30			19.6	578
0230	23.9	4.4	25			26.5	382
0300	26.8	5.4	36			25.0	500
0330	20.0	8.3	34			13.7	470

subject no. 24 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0400	20.4	6.0	36	No Samples		23.0	402
0430	24.7	5.1	44			32.8	392
0500	21.3	5.6	50			33.8	510
0530	27.0	5.0	40			30.4	461
0600	22.2	5.5	36			26.5	314
0630	22.4	6.0	38			34.8	348
0700	22.0	6.3	44			28.4	598
0730	20.4	5.8	33			42.1	402
0800	19.4	5.3	38			34.3	431
0830	19.2	5.2	30			28.4	451
0900	21.9	5.4	34			26.5	402
0930	18.4	4.4	30			24.0	470
1000	20.9	4.8	36			28.9	461

Subject No. 25

Age: 42 Height: 163 cm Weight: 64 Kg % Ideal Weight + 16.6%

Years since ovariectomy: 2

Indication for ovariectomy: Endometrial polyp ? malignant removed at time of curettage. However no malignancy identified in hysterectomy specimen- only secretory endometrium.

Symptoms: Flushes and superficial dyspareunia

Date of study: 20-22/8/76
Dexamethasone treatment: 2mg 2400h 21/8/76

Exact time of flushes: 21/8/76 0015; 0130; 0310; 0700; 0830; 0915; 1031; 1240; 1410; 1506; 1616; 1705; 1800; 1830; 1935; 2105; 2150; 2240; 2325 (hours)
22/8/76 1130 (hours)

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2200	8.9	2.5	<10	93.3	10.5	31.9	353
2230	7.3	5.0	"	170.7	19.0	31.9	206
2300	7.5	2.4	13	133.3	13.0	29.4	480
2330	7.5	4.0	13	93.3	15.5	33.3	412
2400	5.6	2.5	10	66.7	12.5	40.2	323
0030	7.9	2.2	13	64.0	12.0	43.1	382
0100	8.0	1.5	10	49.3	12.0	23.0	353
0130	8.0	2.5	10	57.3	9.5	24.5	348
0200	7.5	3.3	17	45.3	7.5	29.4	245
0230	7.0	2.5	16	52.0	7.0	29.4	274
0300	8.8	1.9	<10	48.0	3.5	27.0	304
0330	8.3	2.9	"	52.0	3.5	31.4	353
0400	7.8	<1.0	-	41.3	3.5	29.9	294
0430	7.8	2.1	<10	64.0	4.5	30.9	314
0500	6.3	1.0	10	54.7	4.0	27.0	314
0530	8.2	2.0	11	90.7	7.0	26.5	294
0600	10.2	1.9	10	133.3	14.0	33.3	289
0630	9.7	2.1	<10	120.0	16.5	27.0	235
0700	8.3	3.9	23	120.0	9.5	29.9	323
0730	7.0	1.7	<10	133.3	-	33.3	314
0800	4.2	3.2	17	101.3	12.5	27.4	333
0830	3.4	2.5	13	114.7	13.0	30.4	333
0900	3.5	<1.0	19	93.3	9.5	29.9	333
0930	4.6	2.2	-	106.7	9.5	29.9	348
1000	4.4	2.2	23	62.7	6.5	35.8	323
1030	5.5	1.8	36	77.3	6.0	36.0	353
1100	8.1	2.1	36	90.7	3.5	33.3	323

subject no. 25 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
1130	4.3	2.2	37	88.0	5.5	31.4	421
1200	6.3	3.1	29	125.3	9.0	39.7	421
2200	3.6	2.9	19	44.0	4.0	34.8	451
2230	6.4	2.5	15	46.7	<3.0	39.7	480
2300	8.9	1.9	21	54.7	"	33.3	451
2330	7.8	6.3	19	44.0	"	31.4	289
2400	9.3	1.7	-	52.0	"	24.5	470
0030	6.8	2.0	11	44.0	"	29.4	392
0100	7.0	5.6	15	42.7	"	31.9	289
0130	7.1	2.7	15	65.3	"	31.4	294
0200	4.5	1.2	19	50.7	"	28.4	402
0230	7.9	<1.0	19	50.7	4.0	28.9	196
0300	7.1	1.7	29	41.3	4.0	30.4	353
0330	8.1	2.0	40	36.0	3.0	28.9	270
0400	7.4	2.1	26	33.3	<3.0	30.4	314
0430	7.0	2.5	29	46.7	"	32.3	500
0500	3.9	1.8	13	37.3	"	26.0	289
0530	2.2	2.6	<10	32.0	"	33.3	402
0600	3.4	1.7	16	34.7	"	31.9	451
0630	1.8	1.6	<10	40.0	"	30.4	412
0700	1.8	1.9	15	36.0	"	31.4	402
0730	1.4	1.8	16	38.7	"	33.3	392
0800	3.6	2.5	13	38.7	"	31.9	451
0830	2.6	1.8	13	29.3	"	33.3	412
0900	1.3	1.5	11	32.0	"	31.9	451
0930	1.3	5.6	17	29.3	"	37.2	304
1000	2.1	2.1	15	<26.6	"	46.1	314
1030	5.2	1.5	15	29.3	"	41.2	304
1100	2.8	2.2	17	32.0	"	40.2	421
1130	7.2	2.2	11	<26.6	"	45.6	333
1200	6.2	2.0	15	"	"	38.2	235

Subject No. 26

Age: 53 Height: 170 cm Weight: 74 Kg % Ideal Weight + 19%

Years since ovariectomy: 15

Indication for ovariectomy: Carcinoma of the cervix

Symptoms: Neither flushes nor superficial dyspareunia

Date of study: 10-12/10/76
Dexamethasone treatment: 2 mg 2400h 11/10/76 & 0.5 mg 0800h 12/10/76

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2300	5.2	3.6	60	106.7	17.5	No Samples	
2330	5.5	3.3	58	61.3	11.5		
2400	6.4	3.0	42	45.3	10.0		
0030	8.8	3.5	32	43.3	7.0		
0100	7.9	3.4	27	53.7	6.0		
0130	8.8	3.7	42	34.7	2.5		
0200	7.1	3.4	33	38.7	3.5		
0230	8.8	2.4	29	38.7	2.4		
0300	8.3	3.2	22	26.6	1.0		
0330	8.4	3.2	51	29.3	1.0		
0400	8.3	2.5	58	34.7	2.5		
0430	5.6	2.8	40	93.3	12.0		
0500	6.8	2.2	55	54.7	5.0		
0530	8.2	1.4	52	66.7	2.0		
0600	7.5	1.4	46	90.7	<1.0		
0630	7.6	2.7	37	65.3	5.5		
0700	6.8	1.3	27	68.0	8.5		
0730	5.9	2.4	31	88.0	20.0		
0800	8.6	2.1	27	77.3	17.0		
0830	N O	S A M P L E		-	-		
0900	7.8	3.2	49	66.7	20.5		
0930	7.7	2.3	40	26.6	15.0		
1000	8.8	2.8	42	50.7	5.0		
1030	5.5	2.6	40	49.3	7.0		
1100	5.4	2.2	51	42.7	3.0		
1130	4.1	2.5	43	34.7	4.0		
1200	6.0	2.0	30	48.0	2.0		
2200	6.6	3.0	80	42.7	3.0		
2230	5.5	2.8	68	46.7	2.0		
2300	5.2	3.3	46	50.7	-		
2330	5.9	2.5	41	<26.6	1.0		
2400	3.7	1.2	58	"	<1.0		
0030	4.1	2.7	54	"	"		
0100	3.5	1.2	42	"	"		

subject no. 26 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0130	4.5	2.4	31	<26.6	<1.0	No Samples	
0200	3.9	2.2	31	"	"		
0230	4.5	3.1	49	"	"		
0300	4.6	3.2	37	"	"		
0330	4.5	1.6	40	"	"		
0400	4.6	3.2	43	"	"		
0430	5.9	3.1	42	"	"		
0500	4.4	3.0	32	"	"		
0530	5.3	2.4	38	"	"		
0600	5.3	2.3	22	"	"		
0630	4.8	2.4	31	"	"		
0700	4.6	2.3	28	"	"		
0730	4.9	2.4	32	"	"		
0800	4.2	1.3	42	"	"		
0830	2.6	2.6	21	"	"		
0900	6.3	2.9	25	72.0	16.0		
0930	7.4	1.6	27	77.3	25.5		
1000	6.7	2.4	23	76.0	20.5		
1030	1.4	2.7	58	80.0	29.0		
1100	3.8	-	40	90.7	28.0		
1130	2.0	3.6	40	96.0	32.0		
1200	1.4	2.9	40	112.0	30.0		
1230	4.0	2.2	60	118.7	32.0		
1300	2.9	3.3	29	122.7	35.5		
1330	2.1	3.2	79	104.0	34.5		
1400	2.8	2.5	62	112.0	38.0		
1430	4.9	3.9	38	101.3	36.0		

Subject No. 27

Age: 50 Height: 157 cm Weight: 58.5 Kg % Ideal Weight: + 11.6%

Years since ovariectomy: 0.3

Indication for ovariectomy: Adenomyosis

Dexamethasone treatment: 1mg 2200h & 0.5mg 0800h commenced on 21/2/77 and continued during study

Date of study: 28/2/77-1/3/77
Androstenedione and cortisol infusion: 0830-1430h during which the subject was lain flat and food but not fluids was withheld

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	2.0	2.3	10	<26.6	<1.0	No Samples	
0030	1.5	2.9	15	"	"		
0100	1.3	2.8	16	"	"		
0130	<1.0	3.1	12	"	"		
0200	"	2.1	16	36.0	1.2		
0230	"	1.6	<10	<26.6	<1.0		
0300	"	2.2	"	30.7	"		
0330	"	-	"	30.7	"		
0400	"	2.3	"	<26.6	"		
0430	1.4	2.4	"	"	"		
0500	1.7	-	"	-	-		
0530	1.3	2.1	"	33.3	<1.0		
0600	1.6	1.9	"	<26.6	"		
0630	<1.0	1.3	"	"	"		
0700	"	2.6	"	"	"		
0730	2.5	2.3	"	"	"		
0800	<1.0	2.1	"	"	"		
0830	"	2.8	"	"	"		
0900	2.7	3.4	12	85.3	8.6		
0930	2.3	2.1	15	144.0	15.5		
1000	1.9	3.2	17	-	22.5		
1030	2.4	4.1	25	186.7	23.0		
1100	4.0	2.8	25	126.7	21.2		
1130	2.2	3.5	19	138.7	25.2		
1200	3.4	2.8	19	118.6	24.2		
1230	2.6	3.7	26	120.1	29.2		
1300	3.7	2.7	27	123.3	25.4		
1330	5.3	3.5	32	170.5	27.2		
1400	5.7	3.0	34	126.7	27.2		
1430	5.3	4.1	32	221.3	26.0		
1500	4.3	2.3	30	92.0	21.8		
1530	4.7	3.2	33	120.0	17.4		
1600	3.7	2.9	21	74.7	14.6		
1630	5.0	3.8	19	101.3	8.4		

subject no. 27 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
1700	1.0	4.1	21	61.3	7.2	No Samples	
1730	"	2.8	18	61.3	6.0		
1800	"	3.4	19	50.7	3.4		
1830	"	3.1	21	44.0	2.0		
1900	1.2	2.5	19	32.0	3.8		
1930	1.0	3.0	17	-	1.0		
2000	"	2.0	18	26.6	"		
2030	"	2.3	19	"	"		
2100	"	1.9	21	32.0	"		
2130	"	1.5	15	26.6	"		
2200	2.2	2.1	16	42.0	"		
2230	1.0	1.8	15	33.3	"		
2300	"	1.4	16	30.7	"		
2330	"	1.6	15	30.7	"		
2400	"	1.7	18	-	"		

Subject No. 28

Age: 49 years Height: 165 cm Weight: 51 Kg % Ideal Weight: - 6%

Years since menopause: 1.0

Dexamethasone treatment: 1mg 2300h & 0.5mg 0800h commencing 3/7/77 and continuing on day of study

Date of study: 9-10/10/77
Androstenedione infusion: 0900-1500h 10/7/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	<1.0	<1.0	<10	<26.6	1.4	-	475
0030	"	2.1	31	36.0	1.2	9.8	575
0100	1.2	2.8	16	<26.6	1.3	11.0	510
0130	1.2	1.9	31	"	<1.0	11.0	475
0200	1.0	2.2	26	"	1.1	8.8	450
0230	1.1	2.4	28	"	1.1	11.2	500
0300	<1.0	2.5	25	"	4.6	12.5	475
0330	"	2.6	30	"	1.5	9.0	575
0400	"	2.8	29	"	<1.0	8.3	575
0430	1.3	2.6	31	"	"	9.0	510
0500	1.1	3.4	39	"	1.6	8.5	550
0530	<1.0	2.6	28	"	1.1	8.3	450
0600	1.0	3.1	29	"	<1.0	11.0	560
0630	1.6	3.4	43	"	1.7	9.0	555
0700	1.7	2.6	40	30.7	1.0	8.5	475
0730	<1.0	3.2	47	<26.6	<1.0	9.0	-
0800	"	3.2	36	36.0	"	9.5	500
0830	1.8	2.8	39	<26.6	"	-	-
0900	<1.0	2.9	38	"	1.1	10.2	555
0930	1.8	3.0	41	134.0	<1.0	8.5	390
1000	1.4	2.6	57	181.0	1.3	9.5	395
1030	1.5	3.2	68	229.0	<1.0	10.5	440
1100	3.2	2.6	80	234.0	1.1	9.8	455
1130	4.1	3.0	70	240.0	<1.0	10.5	510
1200	2.9	2.6	75	245.0	"	11.0	500
1230	3.5	2.6	43	266.0	"	10.8	500
1300	4.3	2.8	68	250.0	"	9.5	510
1330	4.5	2.4	75	173.0	"	9.5	420
1400	4.6	2.5	92	145.0	"	9.3	440
1430	4.8	2.5	67	181.0	"	11.0	460
1500	7.1	2.9	125	208.0	1.1	9.8	440
1530	5.3	3.4	79	112.0	1.1	11.8	510
1600	<1.0	3.9	73	100.0	<1.0	12.5	540
1630	"	3.6	86	71.0	1.2	9.5	435
1700	"	2.4	61	44.0	<1.0	9.8	485
1730	1.3	2.7	46	40.0	-	9.0	440

subject no. 28 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
1800	2.3	3.2	63	<26.6	<1.0	11.3	415
1830	<1.0	2.8	56	32.0	"	11.0	470
1900	1.7	3.0	56	<26.6	"	12.5	550
1930	1.6	2.4	62	31.0	"	11.0	500
2000	1.9	2.2	55	33.0	"	9.5	450
2030	1.4	2.7	47	50.0	"	12.0	500
2100	<1.0	2.1	43	<26.6	"	-	510
2130	1.9	1.7	47	"	"	10.5	470
2200	2.7	1.8	40	40.0	"	9.8	505
2230	1.7	3.0	50	<26.6	"	10.8	555
2300	1.8	1.8	52	"	"	11.0	540
2330	3.3	1.8	49	"	"	9.8	544
2400	1.1	2.1	41	"	"	-	455

Subject No. 29

Age: 55 years Height: 158 cm Weight: 54 Kg % Ideal Weight: + 5.4%

Years since ovariectomy: 8

Indication for ovariectomy: Carcinoma of the cervix

Dexamethasone treatment: Commenced 10/3/77 (1mg at 2400h & 0.5mg at 0800h)

Date of study: 17-18/3/77 (dexamethasone continued) - testosterone infusion 0900-1500h 18/3/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2400	2.9	2.0	<10	<26.6	<1.0	No	Samples
0030	3.4	2.3	"	"	"		
0100	<1.0	3.1	"	"	"		
0130	"	2.5	"	"	"		
0200	"	2.1	"	"	"		
0230	"	1.8	"	"	"		
0300	"	2.2	"	"	"		
0330	"	2.6	"	"	"		
0400	"	1.7	"	"	"		
0430	"	2.7	"	"	"		
0500	"	2.2	"	"	"		
0530	"	<1.0	"	"	"		
0600	"	1.5	"	"	"		
0630	"	1.7	"	"	"		
0700	"	2.0	"	"	"		
0730	2.6	1.3	"	"	"		
0800	<1.0	1.5	"	"	"		
0830	1.8	2.2	"	"	"		
0900	1.8	2.3	"	"	"		
0930	2.0	1.7	107	"	"		
1000	3.7	2.1	153	"	"		
1030	4.5	2.6	188	"	"		
1100	4.6	1.8	182	"	"		
1130	4.1	2.4	167	"	"		
1200	3.1	2.3	172	"	"		
1230	4.4	1.9	207	"	"		
1300	4.8	1.9	186	"	"		
1330	2.2	1.9	199	"	"		
1400	3.6	1.8	218	"	"		
1430	1.6	2.7	204	"	"		
1500	3.1	<1.0	120	"	"		
1530	3.5	1.5	74	"	"		
1600	2.7	<1.0	50	"	"		
1630	2.7	1.8	38	"	"		
1700	4.0	1.7	45	"	"		
1730	3.6	2.0	35	"	"		
1800	2.4	1.8	30	"	"		

subject no. 29 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
1830	2.7	1.7	24	<26.6	<1.0	No	Samples
1900	2.9	2.0	17	"	"		
1930	2.4	1.8	16	"	"		
2000	1.9	1.0	<10	"	"		
2030	1.1	<1.0	"	"	"		
2100	2.8	1.1	"	"	"		
2130	<1.0	2.0	"	"	"		
2200	3.3	1.5	"	"	"		
2230	-	1.1	"	"	"		

Subject No. 30

Age: 51 Height: 160 cm Weight: 73 Kg % Ideal Weight: + 37%

Years since menopause: 0.6

Dexamethasone treatment: 1mg 2300h & 0.5mg 1100h commencing 23/3/78 and continuing on day of study

Date of study: 30-31/3/78
Testosterone infusion: 1400-2000h 30/3/78

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1000	<2.0	2.3	30	<26.6	<2.5	No Samples	
1030	"	2.6	22	"	"		
1100	"	2.1	40	"	"		
1130	2.4	2.2	25	"	"		
1200	2.4	3.1	25	"	"		
1230	2.4	2.7	25	"	"		
1300	2.4	2.4	25	"	"		
1330	3.9	3.0	27	"	"		
1400	4.7	2.3	30	"	"		
1430	4.6	4.2	158	"	"		
1500	4.4	3.8	138	"	"		
1530	3.3	4.5	154	"	"		
1600	4.3	4.8	194	"	"		
1630	5.1	3.6	196	"	"		
1700	4.5	4.2	168	"	"		
1730	<2.0	4.2	182	"	"		
1800	3.0	3.8	195	"	3.5		
1830	<2.0	3.2	190	"	<2.5		
1900	5.5	3.6	210	"	"		
1930	2.0	3.5	208	"	"		
2000	3.9	3.5	190	"	"		
2030	2.0	2.8	90	"	"		
2100	<2.0	1.8	90	"	"		
2130	2.3	2.3	70	"	"		
2200	3.4	1.8	64	"	"		
2230	-	1.8	64	"	"		
2300	4.0	2.7	50	"	"		
2330	4.1	2.2	78	"	"		
2400	2.0	2.5	68	"	"		
0030	4.5	2.7	68	"	"		
0100	5.1	2.7	64	"	"		
0130	2.0	1.7	42	"	"		
0200	<2.0	3.2	44	"	"		

Subject No. 31

Age: 50 Height: 163 cm Weight: 60 Kg % Ideal Weight: + 11%

Years since ovariectomy: 15

Indication for ovariectomy: carcinoma of the cervix

Dexamethasone treatment: 1mg 2400h & 0.5mg 0800h commencing 5/5/77

Date of study: 13-14/5/77
Testosterone infusion: 0930-1530h 13/5/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0300	1.2	1.3	<10	<26.6	<1.0	No Samples	
0330	1.4	2.4	"	"	"		
0400	1.8	1.9	"	"	"		
0430	1.7	1.4	"	"	"		
0500	1.2	2.0	"	"	"		
0530	1.7	1.8	"	"	"		
0600	1.8	2.3	"	"	1.6		
0630	1.3	-	"	"	<1.0		
0700	1.4	-	"	"	"		
0730	<1.0	2.4	"	"	"		
0800	"	2.3	"	"	"		
0830	"	1.9	"	"	"		
0900	"	2.6	"	"	"		
0930	"	1.4	"	"	"		
1000	"	<1.0	92	"	"		
1030	"	"	124	"	"		
1100	"	1.7	130	"	"		
1130	2.5	1.4	128	"	"		
1200	3.3	1.8	154	"	"		
1230	3.2	1.8	173	29.3	"		
1300	3.3	2.3	149	45.3	"		
1330	3.2	1.9	152	40.0	"		
1400	4.1	2.4	188	33.3	"		
1430	4.7	1.9	158	37.3	"		
1500	6.1	3.7	177	46.7	"		
1530	6.5	2.9	124	53.3	"		
1600	3.0	1.7	64	49.3	"		
1630	3.0	1.7	28	28.0	"		
1700	4.8	1.9	34	46.7	"		
1730	6.8	2.3	38	41.3	"		
1800	6.1	1.8	32	30.0	"		
1830	5.5	2.8	22	<26.6	"		
1900	7.4	1.8	22	34.7	"		
1930	7.2	2.6	<10	<26.6	"		
2000	6.8	<1.0	"	"	"		

subject no. 31 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2030	7.0	<1.0	<10	46.7	<1.0	No Samples	
2100	6.9	"	"	<26.6	"		
2130	7.3	-	"	"	"		
2200	6.2	-	"	"	"		
2230	5.9	<1.0	"	"	"		
2300	2.8	"	"	"	"		
2330	1.7	1.3	"	"	"		
2400	<1.0	1.0	"	"	"		
0030	3.6	-	"	"	"		
0100	3.7	-	"	"	"		
0130	3.5	1.9	"	29.3	"		
0200	2.5	1.5	"	<26.6	2.3		
0230	2.0	1.5	"	"	<1.0		
0300	3.7	2.0	"	"	"		

Subject No. 32

Age:54 Height: 155 cm Weight: 58 Kg % Ideal Weight: + 17%

Years since ovariectomy: 12

Indication for ovariectomy: Carcinoma of the cervix

Dexamethasone treatment: commenced one week prior to study and continued on day of study at a dose of 1mg 2400h & 0.5mg 0800h

Date of study: 17-18/3/78
Oestradiol infusion: 1430-2030h 17/8/78

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1030	<2.0	2.0	<10.0	<26.6	N.S.	7.6	490
1100	"	2.0	"	"		7.3	410
1130	"	2.0	"	"		6.0	400
1200	"	1.1	"	"		6.5	395
1230	"	1.6	"	"		7.0	390
1300	"	1.0	"	"		6.0	490
1330	"	1.2	"	"		7.0	520
1400	"	1.6	"	"		8.0	515
1430	"	<1.0	"	"		6.3	420
1500	"	7.4	No Samples			6.5	425
1530	"	14.7				6.8	390
1600	"	-				6.5	400
1630	4.4	18.0				8.0	435
1700	4.4	16.3				6.3	470
1730	2.0	21.6				6.0	320
1800	5.2	-				6.3	385
1830	3.7	17.4				6.3	395
1900	5.7	15.1				5.0	420
1930	4.1	15.4				4.6	380
2000	3.1	15.4				3.8	435
2030	<2.0	15.4				5.5	410
2100	"	15.8				4.8	380
2130	"	8.2				4.8	385
2200	4.2	4.9				4.0	365
2230	2.0	3.8				3.3	415
2300	3.4	3.8				6.5	360
2330	<2.0	2.4				5.3	405
2400	"	3.2				5.0	395
0030	"	1.6				3.8	485

Subject No. 33

Age: 56 Height: 154 cm Weight: 54 Kg % Ideal Weight + 8%

Years since menopause: 6

Dexamethasone treatment: 1 mg 2400h & 0.5mg 1100h commencing 27/8/77 & continuing on day of study

Date of study: 3/9/77
Oestradiol infusion: 0930-1530h

Hormone concentrations:

Time	E_1	E_2	T A F	LH	FSH
0500	<1.0	<1.0	NO SAMPLES	10.3	525
0530	1.4	"		10.3	540
0600	1.2	"		11.0	500
0630	<1.0	"		9.8	475
0700	2.0	"		10.5	550
0730	3.0	"		10.3	525
0800	3.1	"		9.5	450
0830	2.0	"		12.0	510
0900	3.5	"		9.0	600
0930	2.3	"		12.3	650
1000	2.4	10.5		9.3	575
1030	6.1	9.7		11.8	590
1100	7.0	10.4		9.4	575
1130	7.4	13.6		11.8	580
1200	5.2	13.1		10.8	490
1230	2.8	12.5		7.8	590
1300	6.3	12.6		7.5	590
1330	4.4	13.5		7.8	495
1400	4.7	14.5		10.0	490
1430	3.6	13.8		4.9	400
1500	3.0	14.4		5.9	360
1530	1.1	14.7		5.0	560
1600	2.0	6.3		6.8	460
1630	<1.0	5.3		7.1	480
1700	"	3.9		6.3	340
1730	"	3.6		6.0	380
1800	"	3.5		7.0	405
1830	"	1.9		7.9	575
1900	"	2.1		9.0	600
1930	"	2.4		6.5	480
2000	"	1.4		6.0	300
2030	"	2.5		6.8	440
2100	"	1.3		9.0	415
2130	"	1.8		6.3	515
2200	"	1.4		8.0	-
2230	"	1.6		9.9	430
2300	"	1.7		7.5	440
2330	"	1.0		9.8	430
2400	"	-		7.5	380

Subject No. 34

Age: 48 Height: 158 cm Weight: 56 Kg % Ideal Weight: + 10%

Years since ovariectomy: 8

Indication for ovariectomy: Fibroids with ovarian cyst

Oestrogen therapy: Prescribed ethinyl oestradiol 50 ug/day for 7 years after pelvic clearance, discontinued 3 months prior to study

Dexamethasone treatment: 1mg 2300h & 0.5mg 1100h commencing 10/3/78

Date of study: 17-18/8/77
Oestrone infusion: 1700-2300h 17/3/78

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1100	2.5	2.5	10	26.6	1.0	7.3	410
1130	1.6	1.3	"	"	"	-	430
1200	3.7	2.8	"	"	"	8.3	385
1230	1.5	2.4	"	"	"	8.0	450
1300	1.0	1.9	"	"	"	-	475
1330	"	2.4	"	"	"	-	430
1400	1.3	1.9	"	"	"	8.8	425
1430	2.4	1.2	"	"	"	8.0	475
1500	3.1	2.2	"	"	"	7.5	465
1530	4.2	1.7	"	"	"	-	435
1600	1.6	1.2	"	"	"	7.5	455
1630	1.8	1.7	"	"	"	-	385
1700	1.0	1.0	"	"	"	7.3	375
1730	6.2	2.2	NO SAMPLES			8.0	430
1800	16.0	2.4				7.3	395
1830	17.0	-				6.1	330
1900	12.0	2.3				8.0	380
1930	11.0	2.0				6.3	385
2000	8.8	3.1				6.5	360
2030	8.5	4.0				-	385
2100	16.8	3.5				-	360
2130	11.7	3.2				7.5	465
2200	16.0	3.6				7.6	415
2230	23.9	2.4				5.8	415
2300	17.9	2.4				8.0	350
2330	6.5	3.3				7.5	450
2400	5.4	2.2				7.1	455
0030	6.5	1.9				8.0	395
0100	2.8	1.0				6.5	400
0130	2.5	2.1				8.0	345
0200	1.9	1.3				8.5	500
0230	1.5	1.5				8.0	435
0300	2.5	2.0				11.0	455

subject no. 34 (cont.)

Time	E_1	E_2	T A F	LH	FSH
0330	1.0	2.0	NO SAMPLES	8.0	540
0400	-	2.5		9.8	340
0430	2.3	1.7		9.8	450
0500	3.1	1.7		8.5	390

Subsequent management: No improvement of climacteric symptoms with oral oestrogen therapy - discontinued 10/11/77
25mg oestradiol implant subcutaneously 21/11/77

Hormone concentrations:

Date	LH	FSH
21/11/77 (pre-implant)	5.1	370
28/11/77	6.4	140
12/12/77	0.5	31
9/ 1/78	1.4	23
20/ 2/78	1.0	80
19/ 6/78	1.4	94

Subject No. 35

Age: 38 Height: 165 cm Weight: 70 Kg % Ideal Weight: + 30%

Symptoms: Flushes but no superficial dyspareunia

Oestrogen therapy: Prescribed various oral preparations but without relief of symptoms. Oral therapy discontinued 28/1/77
Oestradiol implant: instilled subcutaneously 23/2/77

Hormone concentrations:

Date	E_1	E_2	LH	FSH
23/2/77	5.0	1.0	5.3	185
28/2/77	11.9	11.1	6.5	190
16/5/77	13.8	5.6	3.5	190
13/6/77	11.9	5.7	2.8	188
27/6/77	11.8	4.1	1.9	405
4/7/77	5.0	5.8	2.2	108
12/9/77	8.0	9.0	1.6	64
10/10/77	7.5	6.8	2.0	59

Subject No. 36

Age: 52 Height: 165 cm Weight: 71 Kg % Ideal Weight: + 31%

Years since menopause: 1.8

Symptoms: Flushes but no superficial dyspareunia (recurred after discontinuing Premarin in October 1976)

Date of first study: 11-12/1/77 - (catheter blocked 0600h - venepunctures at 0600h, 0800h, 1000h)
oestradiol benzoate injection: 1mg i.m. 1400h 11/1/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1000	2.6	5.2	46	114.7	9.3	8.0	500
1030	3.6	6.0	44	98.7	7.9	8.0	525
1100	5.3	5.3	39	61.3	9.6	8.0	470
1130	4.7	4.8	48	68.8	8.3	9.3	500
1200	3.1	5.0	39	72.0	6.0	9.4	-
1230	5.1	5.7	33	70.7	8.4	8.0	550
1300	5.4	-	43	78.7	7.6	7.5	500
1330	4.6	5.7	46	85.3	10.1	10.0	585
1400	4.3	6.5	48	68.0	9.0	4.5	450
1430	5.2	5.8	40	64.0	8.8	9.4	280
1500	5.0	7.1	-	101.3	10.2	6.4	540
1530	5.7	10.3	37	86.7	12.1	8.8	600
1600	7.1	-	56	66.7	8.2	1.3	540
1630	8.0	14.2	50	90.7	11.0	14.5	540
1700	9.6	15.5	44	113.3	6.2	8.0	550
1730	10.2	11.9	52	82.7	6.2	7.0	500
1800	7.6	13.3	51	92.0	8.5	7.0	510
1830	8.3	16.1	44	98.7	6.4	6.3	490
1900	7.7	14.6	44	130.7	12.9	1.8	495
1930	8.4	21.2	50	109.3	12.0	7.9	500
2000	5.8	16.2	42	82.7	10.4	1.8	450
2030	6.5	13.4	37	65.3	6.7	5.8	460
2100	8.3	18.3	34	44.0	7.1	5.9	465
2130	10.2	20.6	30	57.3	3.7	4.3	450
2200	9.2	22.9	33	52.0	3.6	4.7	430
2230	9.2	19.1	29	45.3	2.6	4.0	400
2300	6.6	21.9	31	67.0	6.2	5.8	465
2330	5.5	18.9	36	58.7	3.1	4.9	525
2400	4.8	18.0	33	52.0	2.9	5.0	405
0030	6.1	20.6	31	65.3	1.6	5.3	410
0100	4.3	18.9	27	61.3	3.4	5.5	415
0130	4.5	21.7	18	80.0	2.5	5.0	340
0200	5.3	23.3	18	61.3	1.8	5.0	358
0230	4.9	16.0	23	82.7	10.0	3.8	350
0300	6.4	29.1	31	56.0	12.7	5.4	375
0330	7.0	25.8	36	114.7	12.2	5.3	340

subject no. 36 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0400	7.1	30.2	25	-	7.0	4.1	385
0430	7.9	25.2	46	128.0	28.2	10.6	460
0500	-	32.2	-	160.0	-	-	-
0530	6.9	33.8	65	176.0	24.9	5.8	380
0600	8.3	36.0	47	122.7	27.2	8.4	445
0630	NO SAMPLE			-	-	-	-
0700	"	"		-	-	-	-
0730	"	"		-	-	-	-
0800	8.1	26.4	22	77.3	19.6	5.4	575
0830	NO SAMPLE			-	-	-	-
0900	"	"		-	-	-	-
0930	"	"		-	-	-	-
1000	7.7	24.4	19	-	-	5.8	-

Date of second study: 5-6/4/77 - 21st day of third treatment cycle

Oestrogen treatment: Ethinyl oestradiol 30 µg/day, commenced on discharge after first study and was taken every evening for 3 weeks in every 4.

Symptoms: This dose of oestrogen relieved the flushes but was associated with withdrawal bleeding.

Oestradiol benzoate injection: 1mg i.m. 1400h 5/4/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1000	6.4	4.3	20	57.3	27.7	1.8	100
1030	6.2	4.3	37	60.0	37.0	1.7	82
1100	6.5	3.4	35	49.3	19.7	1.4	76
1130	7.8	4.5	31	50.7	11.9	1.8	80
1200	8.3	5.3	40	54.7	15.5	1.5	62
1230	7.3	3.6	28	50.7	17.0	1.3	66
1300	7.0	4.0	39	26.7	14.0	1.3	85
1330	5.4	5.6	48	95.0	15.5	1.3	69
1400	6.0	4.7	37	133.3	17.6	1.2	80
1430	5.3	9.0	37	85.3	16.1	1.2	87
1500	3.3	13.6	36	74.7	11.1	1.6	75
1530	3.9	16.1	36	128.0	14.8	1.6	68
1600	3.2	19.8	37	144.0	13.5	1.4	83
1630	4.7	22.5	41	66.7	10.0	1.5	83
1700	5.0	27.3	34	68.0	17.7	-	-
1730	5.7	31.4	53	70.7	14.5	1.4	74
1800	6.1	37.8	40	98.7	11.9	1.2	80
1830	8.1	36.8	34	58.7	13.0	1.5	79
1900	7.0	43.5	43	48.0	20.4	1.1	72
1930	8.8	31.7	48	50.7	10.6	1.6	76
2000	7.8	45.8	45	38.0	8.3	1.3	75
2030	7.4	49.2	55	38.6	8.9	0.9	82
2100	10.2	51.6	40	45.3	8.5	0.9	83
2130	8.5	51.0	50	30.7	6.0	0.5	73

subject no. 36 second study (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
2200	8.9	49.6	46	44.0	5.5	0.8	70
2230	10.8	49.3	48	82.7	10.6	1.0	72
2300	8.5	52.6	46	78.7	11.8	0.7	66
2330	9.6	52.1	43	61.3	8.4	0.6	69
2400	10.1	48.5	36	50.7	13.9	0.9	66
0030	15.1	50.8	43	58.7	7.0	0.9	68
0100	13.3	49.1	32	49.3	5.0	0.9	69
0130	16.5	52.8	24	106.7	4.8	0.7	63
0200	12.0	53.9	34	122.7	14.2	1.0	65
0230	10.9	56.7	26	68.0	10.6	0.9	52
0300	13.3	57.1	34	93.3	13.3	1.0	57
0330	10.5	52.2	31	93.3	17.5	1.1	64
0400	9.3	46.4	38	130.7	19.0	0.8	55
0430	6.4	50.6	32	77.3	15.7	1.0	57
0500	7.7	54.0	39	133.3	25.6	1.1	57
0530	6.9	40.5	46	90.7	35.0	0.8	57
0600	8.6	51.9	45	120.0	27.7	0.9	51
0630	7.4	40.4	40	221.3	31.2	1.2	50
0700	8.3	32.0	56	208.0	35.0	1.4	60
0730	7.3	40.9	51	162.7	27.2	1.2	35
0800	9.2	33.8	48	93.3	34.0	1.7	56
0830	10.1	37.2	35	72.0	32.2	1.4	68
0900	9.6	31.2	24	45.3	29.1	1.9	54
0930	12.0	36.9	29	41.3	29.7	1.2	64
1000	13.0	30.1	28	53.3	25.0	2.2	60

Subject No. 37

Age: 33 Height: 158 cm Weight: 51 Kg % Ideal Weight:0%

Years since ovariectomy: 2

Indication for ovariectomy: endometriosis

Oestrogen therapy: Oestradiol valerate 1mg prescribed cyclically for 0.5 years - studied on first day of new treatment cycle

Date of study: 29-30/11/76
Oestrogen administered: Oestradiol valerate 1mg 1900h 29/11/76

Hormone concentrations:

Time	E_1	E_2
1500	5.3	2.8
1530	3.9	2.2
1600	No Sample	
1630	5.6	1.8
1700	5.7	1.9
1730	6.5	3.7
1800	5.9	1.7
1830	5.9	3.0
1900	6.9	2.2
1930	5.1	2.0
2000	6.3	3.2
2030	6.8	3.6
2100	11.7	3.2
2130	11.1	4.1
2200	10.5	5.4
2230	11.6	3.7
2300	10.2	4.1
2330	10.5	4.2
2400	10.5	4.3
0030	15.2	4.0
0100	12.9	4.7
0130	10.2	3.6
0200	6.0	3.8
0230	11.7	3.8
0300	9.6	3.9
0330	7.7	4.0
0400	8.6	4.2
0430	7.0	4.3
0500	8.0	4.3
0530	-	4.2
0600	6.6	4.6
0630	6.7	4.4

subject no. 37 (cont.)

Time	E_1	E_2
0700	11.3	4.1
0730	11.2	4.1
0800	6.6	4.2
0830	8.7	4.2
0900	6.6	4.1
0930	5.7	4.4
1000	7.2	4.4
1030	6.8	4.5
1100	6.8	4.4
1130	7.9	4.2
1200	6.8	4.5
1230	8.6	4.4
1300	7.4	4.3
1330	7.6	4.3
1400	6.0	3.8
1430	5.6	4.6
1500	6.8	4.5

Subject No. 38

Age: 56 Height: 155 cm Weight: 57.5 Kg % Ideal Weight: + 15%

Years since menopause: 3.4

Oestrogen therapy: Oestradiol valerate 2mg cyclically for 1.5 years In the 6 months prior to study had developed withdrawal bleeding. Studied on Day 17 of 3 week cycle prior to diagnostic curettage operation which subsequently revealed fibroids and adenomatous hyperplasia

Date of study: 30/6/76-1/7/76
Oestrogen administered: Oestradiol valerate 2mg 1030h 30/6/76

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0930	44.5	9.5	47	80.0	24.9	34.8	270
1000	51.9	8.5	38	48.0	20.9	39.2	461
1030	30.9	7.7	30	48.0	-	38.7	319
1100	42.0	9.3	35	54.7	13.3	41.7	519
1130	44.3	15.6	28	58.7	9.3	41.7	568
1200	60.7	13.5	35	48.0	7.6	37.2	578
1230	80.7	14.3	30	38.7	6.6	42.6	441
1300	54.4	14.8	32	61.3	6.0	28.4	353
1330	54.1	13.3	38	77.3	7.7	27.9	230
1400	62.4	13.6	30	42.7	6.5	32.3	235
1430	46.9	12.2	32	54.7	6.0	24.0	319
1500	53.9	10.9	35	54.7	6.0	27.0	211
1530	67.7	10.3	38	57.3	6.7	23.0	323
1600	67.2	10.5	33	85.3	-	27.4	245
1630	67.5	11.9	30	64.0	21.0	21.1	304
1700	37.6	10.9	33	74.7	-	25.5	289
1730	43.7	10.0	38	120.0	-	24.5	314
1800	72.5	9.6	30	68.0	-	29.9	515
1830	65.6	9.7	33	89.3	11.9	21.1	353
1900	59.7	9.6	38	106.7	19.2	28.9	294
1930	79.3	12.5	38	80.0	14.4	25.5	382
2000	62.9	8.9	38	61.3	8.9	24.5	353
2030	53.4	13.3	35	58.7	8.7	24.0	431
2100	52.3	12.5	28	50.7	8.3	35.3	353
2130	59.4	10.9	35	68.0	6.6	26.5	461
2200	70.8	11.2	32	44.0	5.1	28.4	515
2230	53.2	11.4	33	44.0	3.2	24.5	431
2300	44.6	9.7	30	34.7	2.4	22.1	539
2330	55.8	11.5	35	50.7	2.2	21.6	696
2400	37.2	11.7	33	44.0	1.9	33.3	250
0030	52.4	11.5	33	68.0	3.2	41.2	304
0100	42.0	7.4	32	41.3	2.5	39.2	289

subject no. 38 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0130	47.3	8.9	32	32.0	1.5	15.2	245
0200	47.7	7.9	35	40.0	2.0	21.1	319
0230	46.5	8.3	30	32.0	1.3	28.9	412
0300	50.3	7.7	33	29.3	2.3	33.3	304
0330	50.1	7.7	30	120.0	12.4	27.4	211
0400	41.9	8.2	43	144.0	21.5	23.5	279
0430	40.7	9.5	38	154.7	27.0	14.7	353
0500	35.7	9.3	40	80.0	17.9	24.0	230
0530	47.7	9.2	33	58.7	-	23.0	372
0600	34.2	9.2	32	64.0	12.6	24.0	245
0630	36.2	8.6	33	69.3	9.2	28.4	348
0700	-	8.3	33	85.3	10.5	24.0	250
0730	32.4	7.8	32	68.0	8.4	22.5	245
0800	34.4	7.8	32	125.3	19.3	25.5	250
0830	N O S A M P L E			-	-	-	-
0900	37.1	7.5	37	-	-	24.5	245
0930	26.1	8.0	-	-	-	20.6	245
1000	18.7	6.9	-	-	-	27.4	235

Pre-Hysterectomy 15/7/76

Time	E_1	LH	FSH
0900	2.1	38	480
0930	4.4	33	420
1000	1.9	39	540
1030	2.0	36	440
1100	5.9	32	545
1130	4.9	31	680
1200	6.0	33	520

Post-Hysterectomy 23/7/76

Time	E_1	LH	FSH
0900	1.3	28	370
0930	2.1	31	460
1000	5.4	33	430
1030	4.5	28	555
1100	5.8	32	250
1130	7.4	30	350
1200	4.2	33	230

Subject No. 39

Age: 56 Height: 173 cm Weight: 107.5 Kg % Ideal Weight = 73%

Years since menopause: 2.1

Symptoms: Flushes (no intercourse)

Date of study: 6-7/1/77

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0930	6.3	4.7	33	106.7	9.6	No Samples	
1000	7.8	4.8	35	106.7	9.8		
1030	8.4	5.0	31	126.7	9.7		
1100	9.8	4.8	33	100.0	6.7		
1130	7.7	5.0	31	118.7	8.9		
1200	8.0	6.0	30	124.0	7.3		
1230	8.3	5.5	31	100.0	8.4		
1300	10.5	6.1	31	77.3	7.0		
1330	8.5	6.3	41	85.3	6.3		
1400	7.4	4.9	40	77.3	4.3		
1430	7.7	5.3	35	56.0	4.2		
1500	7.8	4.1	33	57.3	6.2		
1530	7.5	4.2	28	58.7	3.9		
1600	7.8	3.9	41	68.0	6.7		
1630	8.5	4.1	35	62.7	3.5		
1700	7.5	4.2	25	56.0	3.5		
1730	7.9	4.2	35	56.0	2.8		
1800	7.5	5.2	35	66.7	1.8		
1830	8.6	-	46	104.0	2.2		
1900	7.7	5.0	44	57.3	2.1		
1930	8.6	3.9	35	57.3	2.7		
2000	8.2	3.7	25	69.3	1.8		
2030	7.4	4.1	35	50.7	2.7		
2100	7.7	4.2	44	58.7	2.7		
2130	7.5	4.2	30	46.7	1.4		
2200	9.8	4.7	44	<26.6	<1.0		
2230	7.6	6.1	31	"	"		
2300	7.4	5.5	59	"	"		
2330	9.0	4.4	35	"	"		
2400	7.9	5.9	22	80.0	"		
0030	10.3	4.5	22	98.7	2.0		
0100	11.4	4.1	30	34.7	2.8		
0130	10.4	3.6	25	66.7	3.6		
0200	11.1	4.2	27	<26.6	2.7		
0230	10.0	4.4	38	56.0	7.2		
0300	11.3	4.6	28	66.7	6.2		
0330	10.8	3.4	25	62.7	5.2		
0400	11.3	3.2	46	<26.6	3.0		
0430	8.1	3.3	33	74.7	6.3		

subject no. 39 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0500	6.8	3.1	35	124.0	15.4	No Samples	
0530	7.8	2.6	31	48.0	9.2		
0600	10.2	3.7	41	62.7	6.7		
0630	8.5	3.6	38	72.0	4.4		
0700	7.4	3.3	15	105.3	11.7		
0730	8.5	4.5	40	154.7	17.5		
0800	9.5	5.1	35	146.7	19.0		
0830	8.6	5.1	15	102.7	13.9		
0900	8.2	4.4	-	82.7	11.0		
0930	-	4.0	28	82.7	9.9		

Subject No. 40

Age: 51 Height: 175 cm Weight: 64 Kg % Ideal Weight: + 3.5%

Years since menopause: 3

Oestrogen therapy: Conjugated equine oestrogens ('Premarin') 1.25mg for 2.5 years, then piperazine oestrone sulphate 1.5mg cyclically
Years of oestrogen therapy: 2.8

Date of study: 10-11/7/76
oestrogen administered: Piperazine oestrone sulphate 1.5mg 1400h 10/7/76 - day 1 of treatment cycle: no symptoms but withdrawal bleeding at completion of treatment cycle

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1000	4.5	8.5	47	No Samples		11.3	323
1030	4.4	8.2	50			12.7	289
1100	4.9	-	52			8.3	186
1130	7.6	9.5	43			-	-
1200	4.8	7.3	52			13.7	216
1230	7.2	7.9	50			15.2	186
1300	7.9	8.5	47			13.7	186
1330	5.1	12.2	53			19.6	191
1400	5.0	7.0	65			22.5	191
1430	8.4	7.1	52			22.5	98
1500	9.4	7.6	75			5.9	98
1530	10.7	7.7	50			9.8	98
1600	10.0	8.6	43			8.8	98
1630	11.7	10.8	41			8.8	98
1700	17.5	11.2	49			10.3	98
1730	16.0	11.8	39			9.3	98
1800	12.7	12.2	39			9.8	98
1830	12.6	7.0	38			6.9	98
1900	30.5	11.1	39			8.3	216
1930	32.9	10.3	43			7.8	289
2000	29.1	8.8	37			7.4	245
2030	30.4	9.1	41			12.3	274
2100	31.6	10.4	45			10.3	201
2130	28,4	13.2	49			7.4	230
2200	32.7	10.7	53			10.8	98
2230	32.9	9.0	65			9.3	98
2300	26.8	11.1	80			9.8	206
2330	27.2	12.0	68			15.7	191
2400	40.3	10.1	45			14.7	98
0030	30.1	10.3	47			18.6	172
0100	36.5	10.4	43			16.2	235
0130	27.9	10.1	37			15.2	216
0200	28.3	10.8	38			22.1	98

subject no. 40 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0230	25.3	11.2	45	No Samples		7.8	235
0300	27.6	12.0	38			6.4	172
0330	28.5	11.4	44			6.4	98
0400	22.8	12.2	38			11.8	206
0430	35.4	12.0	37			6.9	230
0500	26.1	11.5	42			9.3	216
0530	26.1	12.4	43			6.4	216
0600	23.0	10.7	38			5.9	206
0630	25.9	9.4	38			5.9	191
0700	20.0	11.2	45			11.3	309
0730	26.2	11.1	45			9.3	309
0800	24.5	13.1	47			10.3	387
0830	24.3	10.4	60			10.8	186
0900	28.6	-	43			8.8	348
0930	15.8	12.4	42			11.3	274
1000	20.8	13.0	49			9.8	191

Subject No. 41

Age: 51 Height: 160 cm Weight: 65 Kg % Ideal Weight + 22%

Years since menopause: 2

Previous years of oestrogen therapy: 0.5

Oestrogen therapy: Oestradiol valerate 1mg prescribed cyclically for flushes, but asymptomatic at time of study which was Day 1 of the 6th treatment cycle.

Date of study: 8-9/10/76
Oestrogen administered: 1230h 8/10/76 - catheter functioned incorrectly after 0100 after which blood samples were only obtained hourly.

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0830	6.5	5.5	NO SAMPLES			7.3	235
0900	5.9	4.1				7.0	245
0930	5.7	4.6				7.5	355
1000	6.4	4.9				7.0	265
1030	6.7	5.3				7.0	275
1100	5.0	5.6				5.0	271
1130	6.2	5.4				7.0	271
1200	6.1	5.2				5.3	260
1230	6.6	8.2				8.3	275
1300	30.5	10.5				8.5	365
1330	34.7	11.2				7.8	310
1400	49.8	11.0				7.3	315
1430	50.7	10.2				5.0	290
1500	51.9	10.8				7.0	330
1530	27.8	10.0				7.5	345
1600	33.4	12.0				8.5	290
1630	28.6	10.7				9.5	245
1700	30.9	10.0				5.0	310
1730	39.5	22.6				5.8	300
1800	47.2	18.5				7.5	230
1830	35.4	23.1				6.3	280
1900	29.1	11.0				7.8	325
1930	38.0	9.9				7.5	265
2000	36.6	10.9				7.3	260
2030	27.9	10.6				7.8	305
2100	36.6	11.1				8.5	245
2130	36.1	8.1				7.5	260
2200	37.5	8.1				6.8	275
2230	32.2	9.5				8.5	275
2300	37.4	9.4				7.3	270
2330	38.8	9.6				8.0	250
2400	44.7	-				6.5	275

subject no. 41 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0030	25.7	-	NO SAMPLES			8.3	290
0100	NO SAMPLE					-	-
0130	22.1	10.2				7.0	320
0230	22.5	8.8				7.8	285
0330	20.2	9.3				7.8	245
0430	34.5	9.0				7.8	300
0530	26.6	8.4				8.0	280
0630	15.6	7.8				7.5	320
0730	11.2	8.3				9.0	315
0830	16.7	8.3				8.8	278

Subject No. 42

Age: 48 Height: 163 cm Weight: 67 Kg % Ideal Weight: + 23%

Years since menopause: 0.9

Symptoms: Flushes but no superficial dyspareunia (no atrophic vaginitis and KPI = 23%)

Date of study: 24-25/7/76
Oestrogen therapy: Piperazine oestrone sulphate 1.5mg 1400h 24/7/76 - subsequent relief of flushes during treatment cycle, no withdrawal bleeding in that cycle although bleeding in next cycle.

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
1000	11.4	9.3	43	133.3	9.0	25.5	206
1030	13.4	9.6	40	90.7	8.0	23.0	211
1100	13.0	8.6	45	120.0	6.0	25.5	176
1130	8.1	8.4	43	154.7	9.0	16.7	314
1200	14.4	9.4	45	-	9.0	22.1	245
1230	12.8	7.1	48	160.0	9.0	31.4	353
1300	15.3	8.4	45	181.3	12.0	25.5	353
1330	15.9	9.6	48	152.0	11.0	23.0	451
1400	8.5	7.3	38	108.0	7.0	18.1	274
1430	11.5	9.0	30	96.0	4.0	24.0	279
1500	12.0	6.9	21	85.3	4.0	19.6	289
1530	14.7	8.8	32	101.3	4.0	27.9	250
1600	18.8	7.5	26	128.0	4.0	25.0	245
1630	20.0	9.1	32	154.7	5.0	25.0	250
1700	27.8	9.9	30	136.0	6.0	26.5	206
1730	26.5	8.0	37	128.0	6.0	27.0	245
1800	24.3	11.8	23	192.0	6.0	29.4	353
1830	28.1	8.3	40	184.0	9.0	27.0	353
1900	31.8	9.3	41	172.0	5.0	30.4	279
1930	38.9	9.5	40	160.0	3.0	29.4	451
2000	32.6	9.9	19	106.7	5.0	27.9	270
2030	31.5	10.6	34	114.7	3.0	28.9	363
2100	30.9	9.8	34	98.7	2.0	27.4	353
2130	36.3	10.3	30	106.7	2.0	25.0	402
2200	25.2	13.0	30	82.7	2.0	32.3	274
2230	23.7	10.2	21	70.7	2.0	31.9	245
2300	20.8	-	26	74.7	2.0	28.9	279
2330	25.2	8.5	23	73.3	2.0	22.5	353
2400	27.3	6.0	22	73.3	1.0	21.1	363
0030	28.0	9.4	19	72.0	2.0	26.0	451
0100	24.9	5.7	30	53.3	1.0	20.6	274
0130	25.9	8.7	34	48.0	1.0	16.7	289
0200	29.7	11.4	22	80.0	1.0	22.5	323
0230	28.8	6.5	24	66.7	2.0	21.1	294
0300	23.7	6.7	22	149.3	7.0	25.5	363

subject no. 42 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0330	20.9	6.7	30	130.7	7.0	20.1	245
0400	23.7	6.6	35	128.0	8.0	22.5	245
0430	27.2	6.7	35	157.3	8.0	20.6	279
0500	17.1	7.4	24	194.7	6.0	23.0	274
0530	14.8	8.8	27	130.7	7.0	16.2	274
0600	15.5	6.7	30	125.3	7.0	27.4	206
0630	19.2	11.5	39	256.0	17.0	26.0	250
0700	15.4	9.2	49	264.0	20.0	23.5	249
0730	12.2	5.8	45	160.0	13.5	27.4	323
0800	13.1	5.2	39	146.7	11.5	26.5	279
0830	15.2	8.4	40	165.3	15.5	27.0	235
0900	16.4	6.2	42	280.0	17.0	32.8	319
0930	13.9	6.6	24	218.7	19.5	30.9	304

Subject No. 43

Age: 48 Height: 170 cm Weight: 73 Kg % Ideal Weight: + 14%

Years since ovariectomy: 0.6

Indication for ovariectomy: Fibroids

Symptoms: Flushes (no intercourse)

Oestrogen therapy: Prescribed oestradiol valerate cyclically 2mg/day
In the cycle after this study the subject experienced no relief of her flushes, and no withdrawal bleeding.

Date of first study: 26-27/2/77 - 21st day of 3rd treatment cycle
oestradiol valerate: 2mg 1900h 26/2/77

Exact time of flushes: 1340; 1520; 1550; 1655; 1735; 1836; 1951; 2033; 2144; 2216; 2445; 2316; 0007; 0110; 0215; 0310; 0355; 0440; 0542; 0645; 0726; 0805; 0900; 0959; 1030; 1115; 1215; 1301; 1333; 1435 (hours)

Hormone concentrations:

Time	E_1	E_2
1500	9.5	6.6
1530	11.5	6.5
1600	9.6	8.3
1630	9.8	7.3
1700	9.7	7.7
1730	11.0	5.8
1800	10.4	7.3
1830	9.8	9.1
1900	7.6	5.9
1930	15.1	5.8
2000	14.3	7.4
2030	17.0	7.3
2100	20.2	9.4
2130	14.1	9.7
2200	15.6	10.2
2230	26.1	9.6
2300	32.5	9.4
2330	17.4	8.8
2400	25.2	10.1
0030	13.7	9.6
0100	11.9	9.7
0130	22.3	8.6
0200	18.2	9.3
0230	10.8	9.4
0300	10.8	5.7
0330	14.5	6.2
0400	11.5	5.0
0430	12.9	5.2
0500	8.5	4.3
0530	10.8	7.3

subject no. 43 (cont.)

Time	E_1	E_2
0600	16.4	5.0
0630	9.8	8.4
0700	15.5	5.7
0730	12.0	8.1
0800	11.3	6.3
0830	6.7	10.1
0900	8.3	8.4
0930	13.9	5.3
1000	6.7	3.6
1030	11.4	8.5
1100	10.3	7.8
1130	8.0	7.2
1200	6.7	8.0
1230	8.4	3.7
1300	6.0	5.4
1330	6.9	5.0
1400	7.5	5.8
1430	4.7	6.6
1500	5.0	5.5

Subject No. 44

Age: 49 Height: 163 cm Weight: 57 Kg % Ideal Weight: + 4.4%

Years since menopause: 1.5

Oestrogen therapy: Requiring oestradiol valerate 2mg cyclically for effective relief of symptoms
Years of oestrogen therapy: 0.9

Date of study: 9-10/10/76
oestrogen administered: oestradiol valerate 2mg 1330h 9/10/76 on day 20 of treatment cycle - no withdrawal bleeding at completion of the cycle

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
0930	19.6	6.9	N.S.	101.3	18.6	No Samples	
1000	-	-		-	15.1		
1030	21.0	5.7		68.0	11.5		
1100	21.3	-		74.7	11.4		
1130	22.8	6.9		66.7	10.0		
1200	21.8	5.2		65.3	7.7		
1230	19.2	4.7		93.3	9.2		
1300	20.6	6.0		109.3	15.0		
1330	19.3	6.3		101.3	13.8		
1400	18.7	5.5		104.0	11.8		
1430	18.1	4.9		106.7	8.3		
1500	18.9	5.9		68.0	6.4		
1530	-	-		-	--		
1600	21.0	6.8		58.7	5.8		
1630	23.0	5.7		53.3	4.4		
1700	24.8	7.5		80.0	5.7		
1730	31.0	7.8		117.3	20.0		
1800	22.7	8.7		125.3	25.3		
1830	26.9	8.1		58.7	5.7		
1900	27.3	9.5		96.0	18.8		
1930	26.8	9.8		72.0	15.5		
2000	30.5	9.7		53.3	12.6		
2030	30.1	9.1		48.0	11.8		
2100	31.8	9.6		52.0	9.2		
2130	32.9	7.8		53.3	7.6		
2200	37.0	7.5		59.0	4.6		
2230	36.8	7.3		45.3	4.1		
2300	34.7	7.1		44.0	3.0		
2330	33.0	7.0		44.0	3.1		
2400	31.2	6.9		56.0	2.1		
0030	38.7	7.5		122.7	17.5		
0100	33.9	7.6		71.3	13.2		
0130	19.7	7.3		48.0	10.3		
0200	24.3	6.8		45.3	9.3		
0230	21.5	7.0		42.7	7.3		

subject no. 44 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0300	21.5	7.8	N.S.	56.0	4.9	No Samples	
0330	18.2	9.5		53.3	4.0		
0400	17.5	6.8		48.0	<1.0		
0430	21.7	8.5		104.0	9.6		
0500	19.0	7.6		77.3	9.2		
0530	17.3	7.5		69.3	14.0		
0600	22.8	7.2		65.3	9.5		
0630	20.1	5.9		114.7	11.5		
0700	23.7	7.5		208.0	23.8		
0730	22.5	5.3		170.7	28.7		
0800	20.8	4.7		141.3	24.1		
0830	20.2	5.5		90.7	15.1		
0900	19.2	5.8		78.7	20.6		
0930	22.2	5.0		96.0	17.4		

Subject No. 45

Age: 54 Height: 158 cm Weight: 54 Kg % Ideal Weight: + 3.7%

Years since menopause: 2.2

Oestrogen therapy: On piperazine oestrone sulphate 3mg for 2 years with complete relief of climacteric symptoms - studied on Day 20 of the cyclical treatment (no withdrawal bleeding)

Date of study: 10-11/10/76
Oestrogen administered: Piperazine oestrone sulphate 3mg 1300h 9/10/76

Hormone concentrations:

Time	E_1	E_2
1000	33.2	9.9
1030	39.2	11.3
1100	42.4	9.4
1130	36.1	13.5
1200	35.6	12.1
1230	42.4	10.0
1300	44.5	11.3
1330	22.0	10.3
1400	59.9	9.6
1430	46.7	10.4
1500	55.1	11.5
1530	59.3	13.2
1600	39.1	10.2
1630	40.3	10.5
1700	45.9	12.0
1730	37.1	11.7
1800	38.2	11.6
1830	56.6	12.1
1900	56.9	12.1
1930	50.8	13.2
2000	53.4	14.0
2030	47.7	15.6
2100	47.2	-
2130	48.1	13.5
2200	46.1	13.1
2230	38.7	12.1
2300	43.5	11.2
2330	47.5	14.2
2400	49.2	12.1
0030	47.3	11.1
0100	46.9	13.8
0130	31.8	13.0
0200	28.8	11.0
0230	35.6	10.3

subject no. 45 (cont.)

Time	E_1	E_2
0300	28.8	11.6
0330	27.7	10.7
0400	28.1	10.4
0430	30.6	-
0500	35.3	12.2
0530	34.7	11.4
0600	28.7	12.7
0630	31.6	9.3
0700	30.4	9.1
0730	40.6	12.5
0800	34.5	10.5
0830	35.3	9.5
0900	35.2	9.7
0930	30.6	9.5
1000	35.8	10.3

Subject No. 46

Age: 50 Height: 155 cm Weight: 61 Kg % Ideal Weight: + 22%

Years since menopause: 1.1

Oestrogen therapy: Prescribed piperazine oestrone sulphate 3mg cyclically but experiencing some nausea towards end of each cycle. Studied on 21st day of 7th course of therapy. No withdrawal bleeding.

Date of study: 26-27/9/76

Oestrogen administered: Piperazine oestrone sulphate 1200h

Hormone concentrations:

Time	E_1	E_2
0800	36.2	11.9
0830	43.0	10.6
0900	39.0	12.8
0930	49.0	13.0
1000	34.3	14.4
1030	46.2	14.4
1100	40.5	13.0
1130	32.4	13.0
1200	47.7	12.6
1230	N O	SAMPLE
1300	48.7	13.4
1330	48.2	13.3
1400	48.7	12.5
1430	49.0	15.5
1500	32.0	14.3
1530	34.9	12.8
1600	45.9	14.3
1630	50.3	17.8
1700	39.4	13.0
1730	45.2	16.5
1800	46.2	16.1
1830	57.8	17.0
1900	45.9	16.3
1930	47.7	17.1
2000	51.9	15.5
2030	45.1	15.7
2100	48.4	15.8
2130	47.7	12.0
2200	48.2	13.4
2230	45.4	14.4
2300	45.2	14.1
2330	46.4	13.9
2400	39.6	12.3
0030	30.6	13.9
0100	28.3	12.4
0130	44.5	14.7
0200	31.2	13.6

subject no. 46 (cont.)

Time	E_1	E_2
0230	34.9	13.6
0300	36.0	14.0
0330	35.3	13.5
0400	32.9	12.6
0430	30.8	13.9
0500	33.6	14.8
0530	30.0	15.2
0600	34.9	14.8
0630	37.1	13.7
0700	37.6	14.3
0730	36.5	14.5
0800	36.2	10.7

Subject No. 47

Age: 56 Height: 160 cm Weight: 56 Kg % Ideal Weight + 5.6%

Years since ovariectomy: 6

Indication for ovariectomy: Menorrhagia

Drug treatment: Thyroxine 0.1 mg t.d.s.

Symptoms: Flushes but no superficial dyspareunia

Date of study: 21-23/3/76

Dexamethasone treatment: 2 mg 2400h 22/3/76

Hormone concentrations:

Time	E_1	E_2	T	A	F	LH	FSH
2200	6.6	4.7	28	55.3	4.5	22.5	432
2230	4.4	5.7	29	42.7	3.5	19.1	432
2300	3.8	3.1	27	36.0	3.5	16.2	254
2330	4.6	4.6	22	32.0	2.5	21.6	412
2400	6.8	7.3	22	16.0	1.5	21.1	784
0030	9.4	4.8	22	17.3	2.0	27.0	515
0100	3.6	4.2	24	13.3	1.5	28.4	413
0130	3.9	7.3	24	21.3	1.5	39.2	588
0200	3.2	6.6	21	10.7	2.0	28.4	760
0230	3.4	3.5	21	20.0	1.5	19.1	358
0300	4.6	6.5	24	53.3	4.5	23.0	529
0330	2.0	5.0	24	32.0	1.0	28.4	333
0400	7.2	4.0	21	40.0	3.5	17.6	245
0430	3.2	4.4	22	62.7	6.0	19.1	333
0500	2.4	4.7	24	104.0	12.5	30.4	451
0530	3.1	8.3	21	74.7	11.0	23.5	461
0600	4.7	8.8	19	62.7	10.5	21.6	353
0630	5.5	6.0	22	109.3	16.0	23.5	470
0700	6.9	-	21	106.7	12.0	21.6	470
0730	6.3	8.6	23	144.0	18.5	22.5	407
0800	2.7	6.2	28	120.0	20.0	18.1	382
0830	<1.0	7.7	24	82.7	17.0	25.5	431
0900	.	9.6	24	60.0	13.5	16.7	407
0930	3.7	7.7	24	52.0	12.5	16.7	392
1000	2.4	5.7	21	30.7	10.5	24.0	421
1400	1.5	3.0	21	37.3	8.0	27.0	387
1800	2.0	5.0	18	46.7	6.0	39.2	686
2200	<1.0	4.4	21	52.0	5.0	28.4	564
2230	"	4.0	19	36.0	3.5	27.0	588
2300	"	3.6	22	29.3	-	44.1	613
2330	3.8	4.7	17	29.3	3.0	46.1	676
2400	4.1	3.6	15	18.7	3.0	34.8	833

subject no. 47 (cont.)

Time	E_1	E_2	T	A	F	LH	FSH
0030	2.4	4.5	21	56.0	2.5	31.9	515
0100	<1.0	4.1	24	21.3	3.0	29.9	598
0130	"	2.3	24	26.7	3.5	20.1	451
0200	"	4.1	18	16.0	3.0	23.5	475
0230	"	3.7	24	<10.0	2.5	20.6	338
0300	"	3.7	22	"	3.0	20.6	323
0330	"	4.8	21	"	2.5	22.5	431
0400	"	5.1	22	10.7	1.5	20.6	294
0430	"	4.4	24	21.3	2.0	19.6	314
0500	"	4.8	18	24.0	2.0	16.7	372
0530	"	2.8	22	21.3	1.0	21.6	392
0600	"	2.2	22	10.0	1.5	21.6	392
0630	"	4.6	21	30.7	<1.0	19.1	387
0700	"	4.1	22	30.7	1.5	21.1	402
0730	"	3.3	22	18.7	2.5	21.1	461
0800	"	4.3	21	10.7	2.0	24.0	529
0830	"	4.0	15	16.0	2.0	22.1	539
0900	1.5	5.1	21	<10.0	<1.0	27.0	480
0930	2.2	3.4	24	"	"	20.6	480
1000	3.8	3.6	22	10.7	2.0	19.1	372

Thyroid function tests: Serum thyroxine 249 nmol/L
Free thyroxine index 244

Subsequent management: Thyroxine dosage halved with considerable relief of symptoms

5/7/76 Serum thyroxine 172 nmol/L
Free thyroxine index 164

6/9/76 Random plasma E_2 1.5 ng/dl

APPENDIX V. RELATION OF PLASMA ANDROSTENEDIONE AND OESTRONE

A positive correlation between androstenedione and oestrone concentrations determined in blood samples obtained about 0900 hours from postmenopausal and ovariectomised women has recently been reported by Pelc et al. (1978). However, there are marked asynchronous fluctuations in the plasma concentrations of androstenedione and oestrone throughout the day (Part 2a & 2c). The aims of this study were to determine whether the plasma androstenedione concentration determined in a blood sample obtained at 0900 hours accurately reflected the androstenedione status of postmenopausal women, and to examine further the relationship between plasma androstenedione and oestrone concentrations.

METHOD

Eleven postmenopausal or ovariectomised women were studied by determining the androstenedione concentration in blood samples obtained at 20-30 minute intervals for 24 hours. The mean 24 hour value was then correlated with the value in the 0900 hour blood sample.

The relationship of the plasma androstenedione and oestrone concentrations determined in a blood sample obtained at 0900 hours was studied in the 11 women described above, and a further 10 women who were not studied for 24 hours. Full clinical and sampling details of all subjects are given in Appendix IV.

RESULTS

The mean 24 hour androstenedione concentrations in the 11 postmenopausal or ovariectomised women, and the androstenedione and oestrone concentrations in the 0900 hour sample obtained

from these women, and the 10 other women are shown in Table V.i. There was a significant correlation ($P < 0.05$) of the mean 24 hour androstenedione concentration, and the concentration in the 0900 hour sample (Figure V.i.). The possible error (within the 95% confidence limits) in the androstenedione concentration in the 0900 hour sample in reflecting the mean 24 hour concentration was 32.4 ng/dl within the range 50-120 ng/dl. The correlation of the androstenedione and oestrone concentrations determined in the blood samples obtained at 0900 hours from the 21 women was not significant (Figure V.ii.).

DISCUSSION

This study has shown that the androstenedione concentration determined in a blood sample obtained at 0900 hours may be an inaccurate estimate of the androstenedione status of postmenopausal or ovariectomised women because the determination has a possible error of 32 ng/dl in reflecting the mean 24 hour androstenedione concentration. This finding was not unexpected in view of the diurnal variation in the plasma androstenedione concentration (Part 2 a). Contrary to the findings of Pelc et al. (1978), there was no correlation in the present study of the plasma androstenedione and oestrone concentrations determined in blood samples obtained at 0900 hours, presumably because there is asynchrony of the fluctuations in the plasma levels of these hormones (Part 2 a) and perhaps also because the rate of conversion of androstenedione to oestrone depends on body weight (Siiteri and MacDonald, 1973; Rizkallah et al., 1975; MacDonald et al., 1978). Although the number of women in this study was

small, the results cast doubt upon the validity of the conclusions of Pelc et al. (1978) that oestrone production in postmenopausal women depends primarily on androstenedione production.

Table Vi Mean ± SD 24 hour plasma androstenedione (A) concentrations in blood samples obtained every 20-30 minutes for 24 hours, and plasma androstenedione and oestrone (E_1) concentrations in blood samples obtained at 0900 hours.

NO	A ng/dl		E_1 ng/dl
	2400h	0900h	0900h
1	66.0 ± 28.0	40.0	6.1
2	94.4 ± 56.2	113.3	5.5
3	64.0 ± 21.0	58.7	5.3
4	120.5 ± 34.6	104.0	8.1
5	65.7 ± 27.3	42.7	5.4
6	-	117.3	6.7
7	-	49.3	4.7
13	52.2 ± 30.5	27.0	2.2
14	52.2 ± 17.6	80.0	3.1
16	-	78.7	3.0
17	93.8 ± 51.7	88.0	14.1
18	48.6 ± 17.9	73.0	4.4
19	-	74.0	4.0
20	-	64.0	2.3
21	-	53.3	7.7
22	-	27.0	1.9
23	-	65.3	2.9
25	-	93.0	3.5
26	-	78.7	3.0
36	85.0 ± 29.3	114.7	5.4
39	74.0 ± 32.0	83.0	8.2

Figure V i. Correlation of mean 24 hour plasma androstenedione concentration in blood samples obtained every 20-30 minutes for 24 hours and plasma androstenedione concentrations in blood samples obtained at 0900 hours. (The 95% confidence limits in the regression figure is placed as two standard errors of the estimate $S_y = Sdy\sqrt{1-r^2}$).

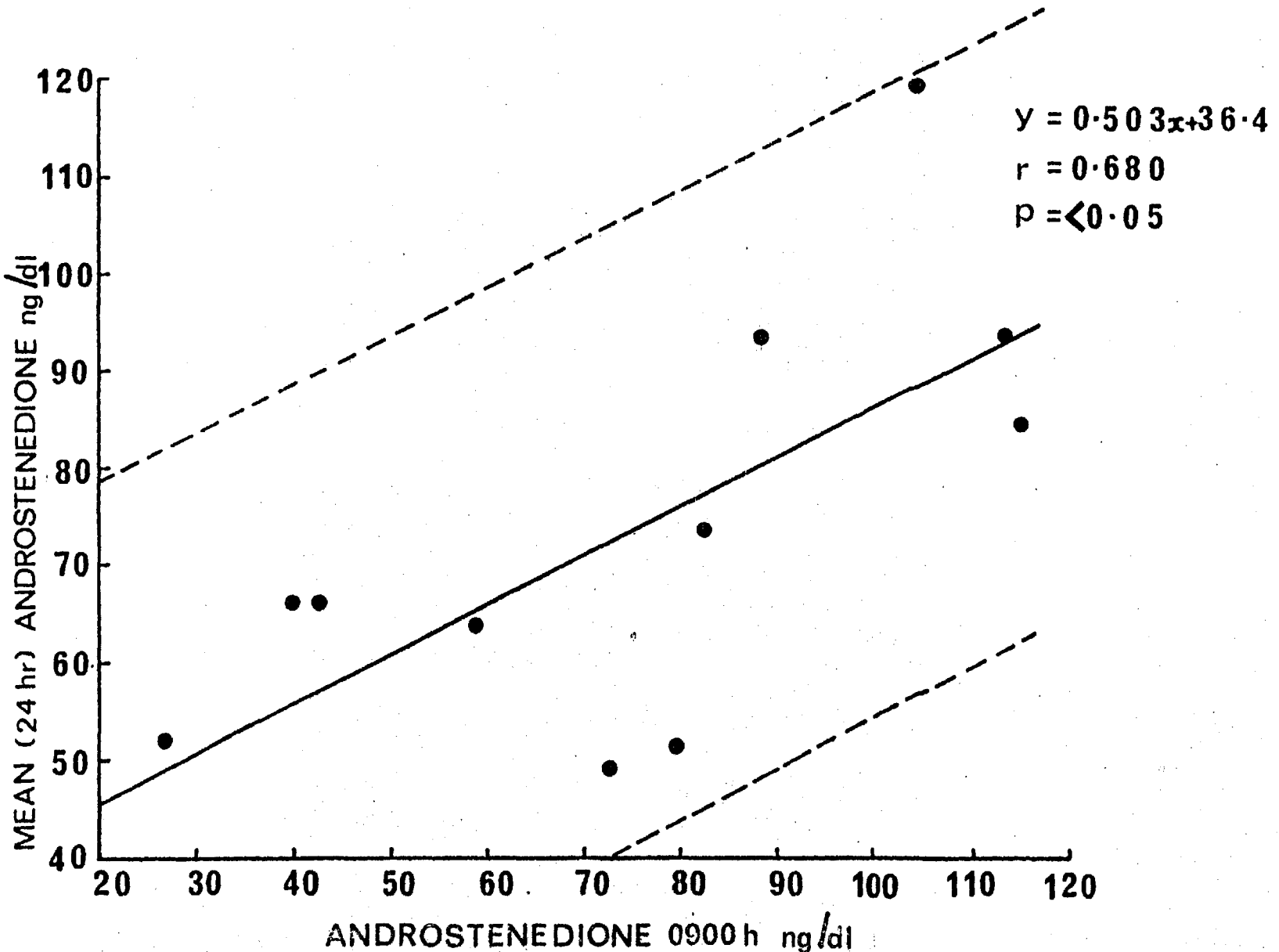
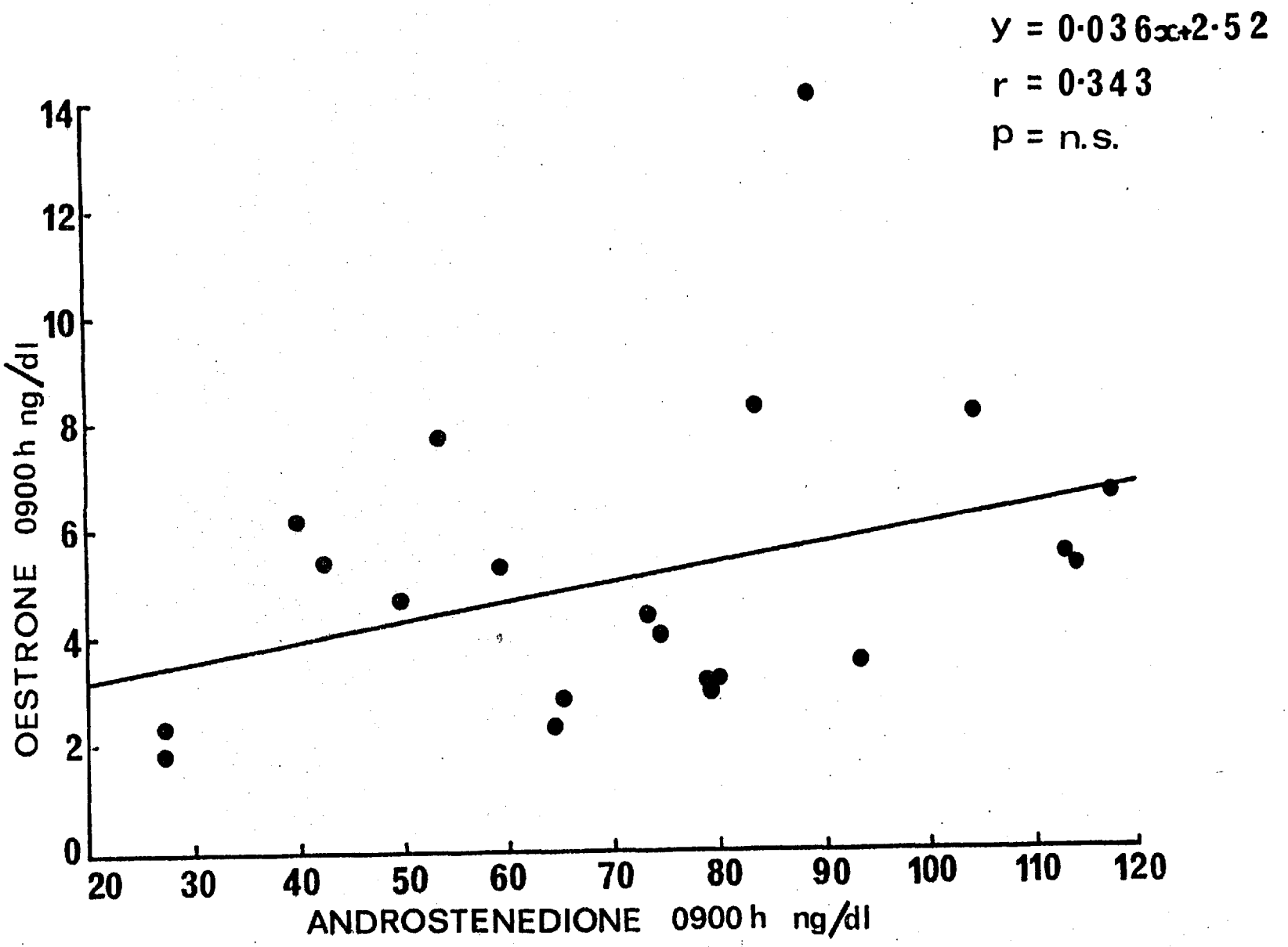

y = 0·503x+36·4
r = 0·680
p =<0·05
MEAN (24 hr) ANDROSTENEDIONE ng/dl
ANDROSTENEDIONE 0900 h ng/dl
120
110
100
90
80
70
60
50
40
20
30
40
50
60
70
80
90
100
110
120

Figure V ii. Correlation of plasma androstenedione and oestrone concentrations in blood samples obtained at 0900 hours from 21 postmenopausal or ovariectomised women.


y = 0·036x+2·52
r = 0·343
p = n.s.
OESTRONE 0900 h ng/dl
ANDROSTENEDIONE 0900 h ng/dl
0
2
4
6
8
10
12
14
20
30
40
50
60
70
80
90
100
110
120