The validity and significance of hormone levels in gynaecology

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Summary

The development of radioimmunoassay of steroid hormones in peripheral venous blood has led to a more direct index of gonadal production and concurrent monitoring of glandular function. While serial analyses of total urinary oestrogens can be correlated with luteinizing hormone (LH) excretion in treating amenorrhoea, radioimmunoassay of plasma oestrogens measures acute changes in ovarian responsiveness. For studies of androgens radioimmunoassay of plasma testosterone is the method of choice, but fractionation of urinary 17-oxosteroids may be useful and can be extended to include the pregnane series and corticoids. Urinary pregnanediol, however, may not reflect the endogenous secretion of progesterone and should be reserved for assessing induction of ovulation. Luteal function can be evaluated rapidly and specifically by radioimmunoassay of plasma progesterone. Although radioimmunoassay offers a new approach to ovarian physiology, its systematic application to disease is still in the future.

DURING recent years the development of ultra-sensitive methods for the determination of steroids in biological fluids has led to two major advances in the clinical application of hormone assays. First, the direct determination of low concentrations of the natural hormones in peripheral venous blood has led to more valid indices of gonadal hormone production. Secondly, the rapidity of modern techniques permits concurrent rather than retrospective monitoring of glandular function. To cite but one example, the choice of the quantity of gonadotrophin used for the induction of ovulation can now be based upon results obtained on the same day that the sample is taken and this minimizes the risk of hyperstimulation and of multiple pregnancy.

This paper is not a review of the vast literature but will be limited to a practical consideration of modern attempts to assess the endogenous production of steroid hormones. The most direct approach is to calculate the blood production rate of hormone in terms of the mean plasma concentration and the metabolic clearance rate of isotopically labelled hormone. Subsequently, the secretion rate of hormone can be calculated from the production rate with a

knowledge of peripheral transformations. Thus, the blood production rate of testosterone in healthy women has been calculated to be 0.34 mg/day and, since approximately 0.20 mg is derived from androstenedione, the secretion rate of the hormone is approximately 0.14 mg/day (Tait and Horton, 1966).

Examples of secretion rates in healthy men and women are shown in Fig. 1. These values represent the current concept of the secretion of these hormones in quantitative terms and are in sharp contrast with some of the parameters used in the past. Thus, whereas there is a major overlap in the range of urinary total 17-oxosteroid excretion between men and women, it will be noted that the secretion rate of testosterone is more than fifty-fold greater in men than in women. However, the calculation of secretion rates involving the administration of more than one labelled steroid is not a practical approach for clinical work and, even if very low amounts of radioactivity are administered, the advisability of giving radioactive steroids to women who may subsequently become pregnant may be questioned. Accordingly, we must consider less complicated ways of obtaining similar information.

FIG. 1. Androgen secretion rates (mg/day): a schematic representation of the results of Tait & Horton (1966).

Indices of oestrogen production in women can be summarized as follows: (1) bioassay; (2) vaginal cytology; (3) urinary oestrogen determination; (4) plasma oestrogen determination; (5) metabolic clearance rate, production and secretion rates.

Bioassay is complicated and expensive and its use is mainly limited to pharmacological studies.

Vaginal cytology when carried out as serial tests may yield useful information—as in monitoring the effect of therapy but the results can also be misleading and are qualitative rather than quantitative.

By far the most established approach is a determination of the total urinary oestrogens (oestrone, oestradiol- 17β and oestriol) although it must be remembered that there are numerous other oestrogens in urine. With regard to this test it should be emphasized that little is to be learned from an isolated determination, and serial analyses are essential. The typical biphasic excretory pattern of the ovulatory menstrual cycle is shown in Fig. 2 and the variation which can occur from day to day in the same post-menopausal woman, in Fig. 3. A useful chemical application involves a correlation between the excretion of LH and total oestrogens in selecting appropriate therapy in infertile women with established amenorrhoea. Values for urinary LH in normal cycles are shown in Fig. 4.

In view of the spectrum of oestrogen metabolites, an attractive alternative is the determination of oestradiol, oestrone or one of the oestriols in peripheral venous blood and results obtained by a radio-

immunoassay (Emment, Collins and Sommerville, 1972) are summarized in Table 1. Plasma analysis has the advantage that acute changes can be observed, for example, the oestrogen surge which may coincide with or precede the LH peak or acute changes in dynamic tests, e.g. in assessing the responsiveness of the ovary to gonadotrophin therapy (Fig. 5). On the other hand, plasma oestrogen analyses have the disadvantage that there may be random or nyctero-hemeral changes from hour to hour although these

FIG. 2. Total urinary oestrogen excretion. Mean levels obtained from eleven normal menstrual cycles.

FIG. 3. Day-to-day variations in the urinary excretion of total and fractionated oestrogens in a post-menopausal woman.

TABLE 1. The mean and range of values for oestrone and oestradiol in peripheral venous plasma from groups of healthy men and women

	Oestrogen pg/ml plasma			
	Oestrone		Oestradiol	
	Mean	Range	Mean	Range
Men	48	29-75	28	13-46
Women				
*Days 1-10	117	45-210	64	18-214
11-18	162	75-240	161	53-340
19-30	156	96-209	132	26-242
Post-menopausal	32	13-56	15	< 10-36

* Day 1 = first day of menses.

FIG. 4. The levels of urinary LH (mean $\pm$ s.d.) in seven subjects (cycle 1).

do not appear to be major in either non-pregnant women (Fig. 6) or during pregnancy.

The study of androgen production in women is complicated by several factors—notably the low concentrations of androgens in blood and tissues, the transformation of androgens to oestrogen and the extent to which androgenic activity may depend upon conversion to other products. Methods which have been used for the assessment of androgen production in women include: (1) bioassay; (2) urinary 17-oxosteroids (total or fractionated); (3) urinary testosterone glucuronoside; (4) plasma testosterone and other C_{19} -steroids; (5) metabolic clearance, blood production and interconversion rates; (6) 5α -reductase activity in growth-responsive tissues.

Bioassay had the obvious advantage that biologically active androgen was determined but proved too complex for clinical application.

The determination of total neutral 17-oxosteroids has serious limitations as an index owing to the fact that this group of compounds is mainly derived from non-androgenic or weakly androgenic precursors and that the principal androgen—testosterone—is not a 17-oxosteroid. On the other hand, useful

FIG. 5. Monitoring the effect of ovarian stimulation by plasma oestradiol determinations.

information can be obtained by the determination of individual 17-oxosteroids and this approach has been extended to the determination of numerous neutral steroids including compounds of the pregnane series and corticoids (Ros and Sommerville, 1971). An example of one of these steroid profiles using high resolution glass capillary columns is shown in Fig. 7, and the results throughout a menstrual cycle are shown in Fig. 8.

FIG. 6. The nycterohemeral variation of oestrone and oestradiol in peripheral venous plasma from a healthy woman on days 22 and 23 of her cycle. ○—○, Oestradiol ●---●, oestrone.

FIG. 8. The level of various C_{19} -steroids throughout a complete menstrual cycle (cycle 1) determined by a high resolution gas-liquid chromatographic procedure.

FIG. 7. The gas-liquid chromatographic separation of a standard mixture (20 ng of each) of authentic steroids using a high resolution glass capillary column.

Reference has already been made to the calculation of testosterone secretion rates and the practicability of such a technique in clinical assessment. The assay of testosterone and epitestosterone glucuronosides in urine has been applied to the investigation of hirsutism and menstrual disorders, for example, by Tucker, Bishop and Sommerville (1969). More recently, radioimmunoassay of plasma testosterone has been the assay of choice although other androgens, such as 5α -dihydrotestosterone, are now being recognized as having at least equivalent importance (Mansfield and Johnson, 1974). The levels of testosterone, determined by the method of Collins *et al.* (1972), found in plasma from healthy women and those with certain pathological conditions are recorded in Table 2.

Indices of progesterone production can be summarized as follows: (1) 5β -pregnanediol and other metabolites in urine; (2) progesterone and 5β -pregnanediol in blood; (3) calculation of production rate; (4) 5α -reductase activity in growth responsive tissues.

The first of these indices, the quantitative deter-

mination of one of the urinary pregnanediols, is the time-honoured approach which has been used for many years, although it is still not clear how closely the excretion of this metabolite may reflect the endogenous level of production of the hormone. There is a characteristic rise in urinary pregnanediol excretion during the ovulatory menstrual cycle, although the extent to which this occurs is rather variable and the difference between levels in the follicular and luteal phase may not be very striking. That this rise is due to extra adrenal precursors was demonstrated by a study after bilateral adrenalectomy (Klopper, Strong and Cook, 1957). If pregnanediol assays are to be used instead of plasma progesterone assays then it is probable that the main field of application is in studying the effect of therapy—notably the induction of ovulation.

Recent advances have made possible the rapid and specific determination of progesterone in peripheral venous blood by radioimmunoassay and results obtained by Youssefnejadian *et al.* (1972) are shown in Table 3. Comparable values are obtained during the luteal phase of the menstrual cycle when the

TABLE 2. Plasma testosterone levels in healthy females and some endocrinological disturbances

	No. of determinations	Testosterone ng/100 ml plasma	
		Mean $\pm$ s.d.	Range
Regular cycles, no hirsutism	46	49 $\pm$ 16	18–133
Regular cycles, with hirsutism	32	118 $\pm$ 36	47–184
Oligomenorrhoea	35	118 $\pm$ 89	23–345
2° Amenorrhoea	14	90 $\pm$ 45	41–143
Stein-Leventhal syndrome	20	135 $\pm$ 60	54–248
Arrhenoblastoma	2	561 & 355	—

TABLE 3. Mean and range of values for plasma progesterone. Day 1 is defined as the first day of menses

	No. of determinations	Progesterone ng/100 ml plasma	
		Mean $\pm$ s.d.	Range
Men 20–40 yrs	72	23.0 $\pm$ 6.8	8–37
Women days 1–14	13	42.4 $\pm$ 18.7	24–79
Women days 15–end of cycle	12	770 $\pm$ 190	90–980
Women* pregnant 12 weeks–term	42	10.4 $\pm$ 5.5	4–23

* μ g/100 ml plasma.

method is performed with or without a chromatographic step and this provides a useful rapid method for the evaluation of luteal function. The difference between the progesterone concentration in the two phases of the menstrual cycle is much more dramatic than the corresponding values for the urinary metabolite and, in view of the simplicity of the technique (one technician can analyse twelve samples by the rapid method in 4 hr), plasma progesterone determinations are recommended in favour of urinary pregnanediol on both practical and theoretical grounds. Furthermore, it should be noted that no significant nycterohemeral variations in progesterone concentrations have been found in the peripheral venous blood of non-pregnant women.

This review has been mainly limited to attempts to assess the production of oestrogens, progesterone and testosterone and space does not permit a consideration of other steroids which may play an important role in human reproduction. A notable example is androstenedione where the secretion rate

in women is greater than in men. This steroid is of especial interest as it is a major precursor of oestrogen production in women and may be the principal source of oestrogens in the post-menopausal woman. In the Stein-Leventhal syndrome androstenedione levels are raised owing to reduced activity of the aromatization enzymes (Savard, Marsh and Rice, 1965). Another steroid of interest is 17-hydroxyprogesterone which may be assayed simultaneously with progesterone and 20 α -dihydroprogesterone (Florensa *et al.*, 1976). An example of the results obtained in ovulatory menstrual cycles is illustrated in Fig. 9.

In conclusion, recent advances in methodology offer the hope of new approaches to the study of ovarian function. It will be obvious from this paper that international research is still mainly concerned with the establishment of steroid concentrations in health and that systematic application to disease is largely a matter for the future.

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FIG. 9. Daily concentrations of progesterone (●—●), 17-hydroxyprogesterone (○--○) and 20 α -dihydroprogesterone (○—○) during a 'normal' menstrual cycle. (—) = mid-cycle L.H. peak.