Journal of Steroid Biochemistry, Vol. 9, pp. 429–436

0022-4731/78/0501-0429$02.00/0

CENTRAL CONTROL OF STEROID HORMONE SECRETION

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SUMMARY

Peripheral plasma levels of cortisol and androstenedione exhibit closely related patterns of change which reflect the episodic secretion of these steroids and their dependence upon corticotrophin. The diurnal pattern appears to be reproducible from day to day within an individual, implying the existence of some type of programmed C.N.S. activity. Although plasma aldosterone levels show changes which also relate closely to those described by cortisol, it appears that it is renin, not corticotrophin, which mediates these changes and thus ACTH and renin release appear to be linked in some way. Stimulation of adrenocortical activity with synthetic corticotrophin, or infusion of androstenedione, illustrates the rapid conversion of this steroid to oestrone. The pattern of peripheral steroid levels observed suggests that the clearance of androstenedione must alter rapidly, and confirms the importance of the adrenal cortex as an indirect source of oestrogen.

Excluding pregnancy, the adrenal cortex and the gonads are the only known sources of steroid hormones in the human. They play a vital role in controlling or influencing a wide range of metabolic processes, including those involved in reproduction. The control of steroid hormone secretion both quantitatively and temporally, needs to be precise, since only relatively minor departures from normality can produce serious metabolic disorders. A clearer appreciation of the nature of these control processes is essential to a full understanding of both normal physiological events, and of pathological disorders.

Our knowledge of these control mechanisms has expanded considerably over recent years, largely because improvements in hormone assay techniques have made it possible to measure hormone concentrations with high specificity and precision in relatively small volumes of blood. Further, by applying these techniques to studies in which sequential blood sampling is carried out at short time intervals, temporal profiles of hormones can be constructed, which have offered new insights into the hormonal changes which occur both in normal subjects and in patients with specific diseases. The combination of these techniques has proved to be a powerful tool in investigative endocrinology. Our own studies have been aimed at improving our understanding of the mechanisms which control steroid secretion, and in this paper, we discuss some of our data and their relevance to our understanding of the problems of adrenocortical physiology.

A number of carefully documented investigations

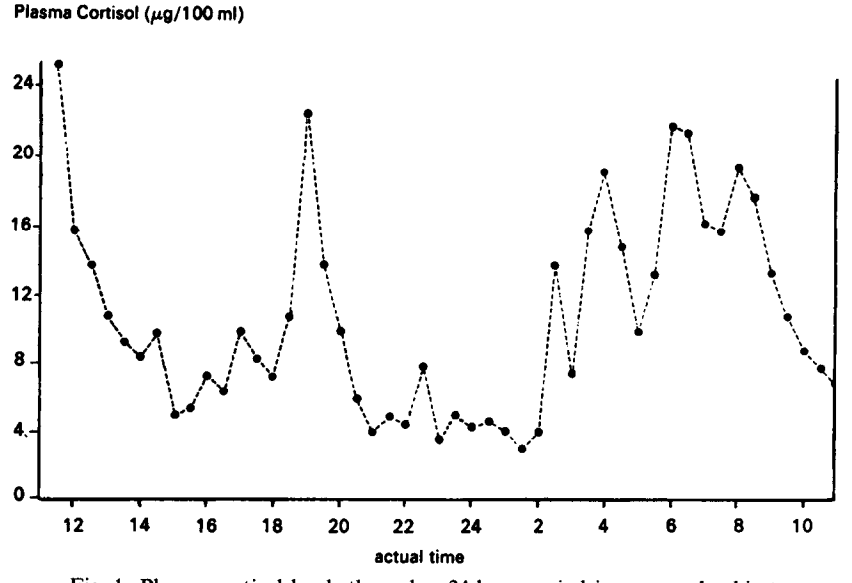

Fig. 1. Plasma cortisol levels through a 24-hour period in a normal subject.

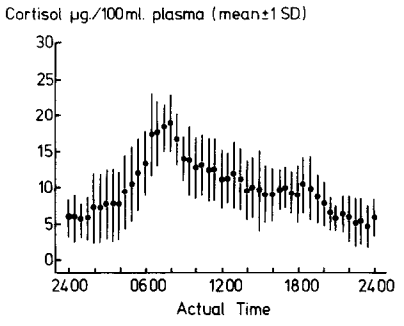

Fig. 2. Mean plasma cortisol levels in a group of 22 normal subjects studied through 24 hours.

have shown that the peripheral blood levels of many steroid hormones exhibit relatively rapid fluctuations, a phenomenon which is ascribed to intermittent hormone secretion. One of the most extensively studied steroids has been cortisol, and early observations by Hellman *et al.*[1], and by Krieger *et al.*[2] established the episodic nature of cortisol secretion and the close correlation between plasma levels of cortisol and corticotrophin (ACTH). These and other studies characterise the normal pattern of cortisol secretion as a number of discrete secretory episodes, usually between 5 and 13, occurring through 24 hours with a major degree of secretory activity arising in the latter part of the sleep period (Fig. 1).

The episodic nature of cortisol secretion stands in strong contrast to the earlier view that steroid hormone secretion under basal conditions exhibited relatively little fluctuation. Although the concept of a diurnal rhythm in secretion was established many years ago, it required the study of the total steroid profile to reveal that this was due to the larger number of secretory episodes which occur during the nocturnal period as compared to the day period, rather than a smooth waxing and waning of cortisol secretion.

The observation of episodic cortisol secretion is not easily accommodated in the concept of negative feedback control of ACTH release by circulating cortisol levels, an hypothesis which has been explored extensively [3]. The postulate that the elevation of plasma cortisol following a secretory episode causes temporary inhibition of ACTH release, followed subsequently by another episode of ACTH release as deinhibition occurs, seems unlikely since there are relatively long periods during which plasma cortisol levels remain low, apparently without stimulating ACTH release. It has also been demonstrated [4] that episodic release of ACTH occurs even when plasma steroid levels remain low for a prolonged period, as in a patient with adrenal insufficiency.

A study of the reproducibility of the pattern of cortisol secretion also suggests that secretion occurs in response to programmed central nervous system control, rather than to changes in peripheral steroid levels. Figure 2 shows the mean plasma cortisol levels in a group of volunteer subjects, studied through the 24 hour period. The picture is broadly similar to that illustrated in the single subject in Fig. 1, illustrating the general similarity of the pattern between subjects. However, even more striking are the results of a study in which the same subject was studied through two sequential periods of 24 hours (Fig. 3), and another in which the studies were made at an interval of two months (Fig. 4). The pattern of cortisol secretion is remarkably similar on the two separate occasions, again suggesting that the secretory pattern is not a random phenomenon, but is pre-programmed, with a considerable degree of reproducibility.

Another major secretory product of the human adrenal cortex is aldosterone. The problem of the nature of the control mechanisms governing the secretion of this hormone has also received considerable attention, and it now seems clear that a number of factors are involved, including the renin-angiotensin system, ACTH, and plasma potassium levels. (See review by Stockigt [5].) However, almost all the data on which our knowledge is based have related to unusual or abnormal conditions, and only recently have

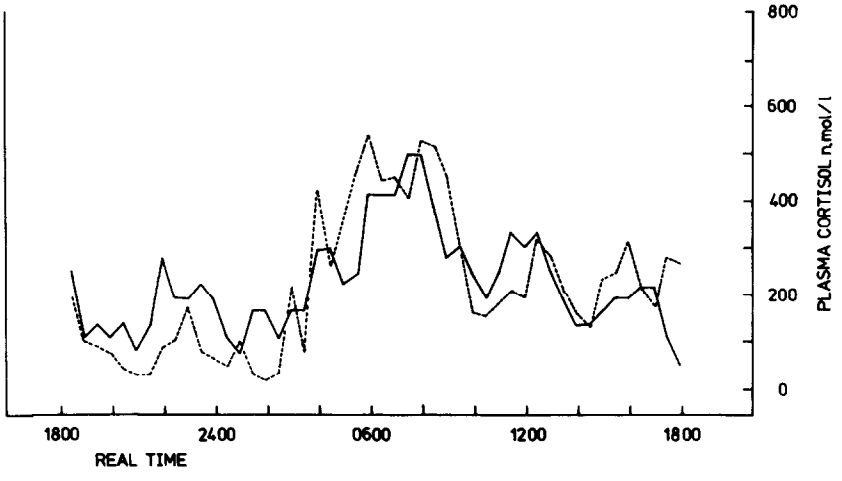

Fig. 3. Plasma cortisol levels through two subsequent 24-hour periods in a normal subject.

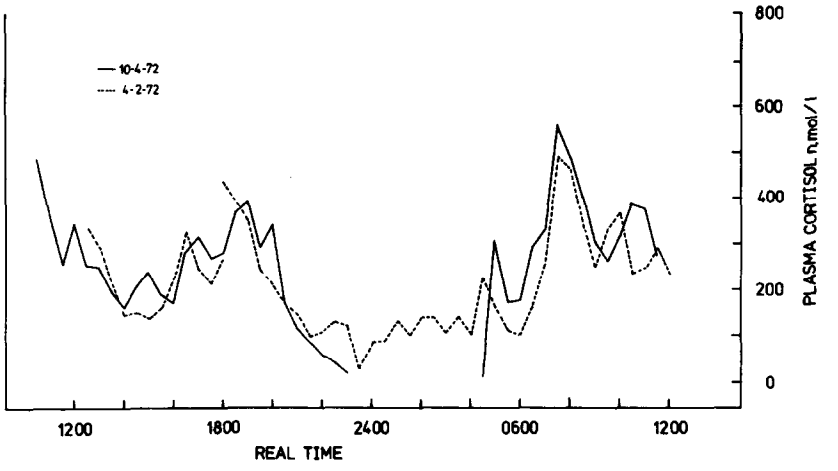

Fig. 4. Plasma cortisol levels through two periods of 24 hours, separated by an interval of two months, in a normal subject.

investigators turned their attention to the nature of basal aldosterone secretion and the mechanisms controlling it.

Studies of plasma aldosterone levels, carried out in control subjects, show that, as with cortisol, aldosterone is secreted episodically. Figure 5 shows the pattern of nocturnal plasma aldosterone levels in a group of normal control subjects. The general pattern is similar to that which is seen for cortisol (Fig. 2), during the nocturnal period, with a zenith at 0700 hours. As we have shown elsewhere [6, 7] there is, in individual control subjects, a remarkably close relationship between the plasma levels of cortisol and aldosterone, and a typical study is shown in Fig. 6. This close correspondence of cortisol (and, by inference, ACTH) and aldosterone levels immediately brings into question the nature of the mechanism controlling plasma aldosterone levels. It seems likely, although not formally demonstrated, that the fluctuating aldosterone levels are reflecting, as with cortisol, episodes of aldosterone secretion by the adrenal cortex. The attractive hypothesis that a common stimulus, namely ACTH, is driving the simultaneous secretion of both steroids appears untenable, since suppression of ACTH release by dexamethasone [6] or elimination of ACTH secretion as in the hypophysectomised subject, [7] fails to prevent episodic secretion of aldosterone.

The cause of the episodic fluctuations in plasma aldosterone is still unclear. They appear not to be related to changes in plasma potassium, and we have not been able to convince ourselves fully that they can be related properly to the renin-angiotensin system. In some cases, where relatively major alterations in plasma renin activity occur, there is a persuasive relationship to plasma aldosterone levels. In many instances though, such a clear relationship does not seem to emerge and this leaves us with the problem of seeking some other mechanism, which, whatever its nature, must in some way be closely synchronised with ACTH release.

In addition to aldosterone and cortisol, the human adrenal cortex secretes quantitatively important amounts of C_{19} steroids, notably dehydroepiandrosterone (DHA) and its sulphate, and androstenedione. These steroids too, exhibit relatively rapid fluctuations in their plasma concentration, under basal conditions.

Rosenfeld *et al.*[8] showed that DHA exhibited a secretory pattern closely similar to that of cortisol,

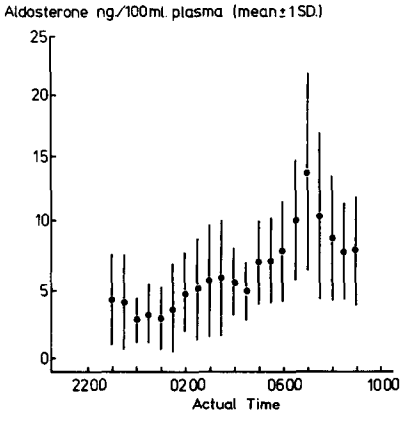

Fig. 5. Mean plasma aldosterone levels in a group of 10 control subjects studied overnight.

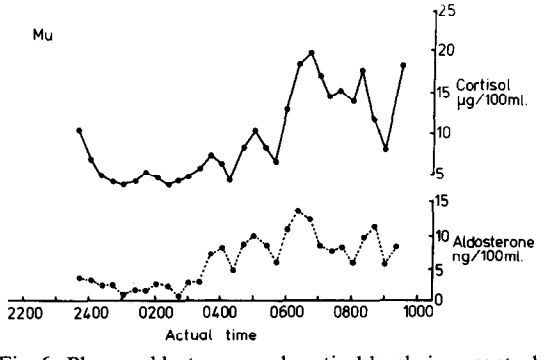

Fig. 6. Plasma aldosterone and cortisol levels in a control subject studied overnight.

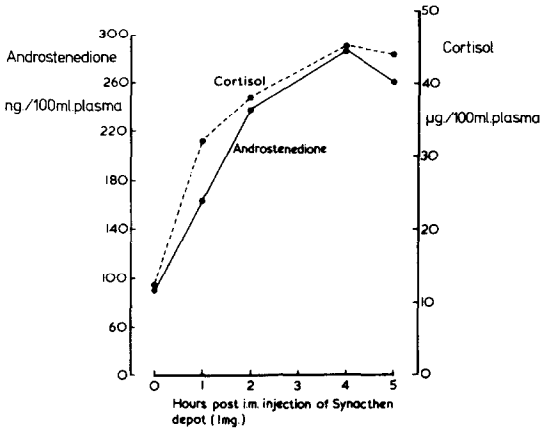

Fig. 7. Effect of ACTH stimulation on plasma cortisol and plasma androstenedione levels in a normal subject.

whilst DHA sulphate levels, although undergoing diurnal changes, did not relate convincingly to cortisol.

Androstenedione secretion is sensitive to ACTH stimulation, as is cortisol, and as Fig. 7 shows, administration of ACTH will cause prompt elevation of both cortisol and androstenedione levels in peripheral plasma. Administration of dexamethasone will also reduce plasma androstenedione levels and thus it is unsurprising that this steroid shows a similar pattern of diurnal change to that seen with cortisol [9]. Figure 8 illustrates in a control subject the close correspondence between levels of the two steroids and is a typical example of the pattern seen in a series of control subjects studied under carefully controlled basal conditions. However, it may be of some significance that occasionally there are minor discrepancies between the plasma levels, and there are periods when androstenedione levels fluctuate independently of cortisol.

In the human female, both the adrenal cortex and the ovaries secrete androstenedione. Elsewhere, we have demonstrated that ovarian secretion of androstenedione can produce major and rapid changes in plasma levels of this steroid in patients with ovarian dysfunction [10]. An explanation of occasional dissociation between cortisol and androstenedione levels in plasma may be that these represent episodes of gonadal secretion. A less likely possibility is that occasionally adrenocortical secretion occurs independently of ACTH stimulation.

Figure 9 illustrates that when adrenocortical activity is suppressed by using dexamethasone to block the secretion of ACTH, in the absence of ovarian function, plasma levels of both cortisol and androstenedione approach the limit of detection. It seems unlikely therefore that the adrenal cortex secretes androstenedione in the absence of any stimulus from ACTH. On the other hand, ovarian secretion of androstenedione is substantial and may occur episodically. Figure 10 illustrates the pattern of plasma androstenedione levels in a patient with long-standing Addison's disease and thus with presumed complete adrenocortical insufficiency. In this patient, the only significant source of C_{19} steroids is the ovary. As the figure shows, plasma levels vary through the 24-hour period and occasional major fluctuations occur. However, although the amount of androstenedione in plasma is well within the range seen in normal control female subjects, illustrating the substantial contribution by the ovaries to the total secretion, the extent of fluctuation is relatively small as compared to the major episodic fluctuations which occur with both cortisol and aldosterone. These small peaks may nevertheless, explain the occasional dissociation noted above between androstenedione and cortisol plasma levels.

These data then, establish that the dominant factor in the control of plasma androstenedione levels, and presumably of androstenedione secretion is apparently ACTH. Since androstenedione is a pre-hormone for both testosterone and oestrone, ACTH is also a significant factor in the regulation of the production of both these hormones and it is pertinent to examine further its role. The procedure adopted has been to examine the plasma steroid profile through a control period to establish the basal pattern, using as subjects, women who had been ovariectomised and thus in whom the complication of ovarian steroid secretion was eliminated. By administration of dexamethasone,

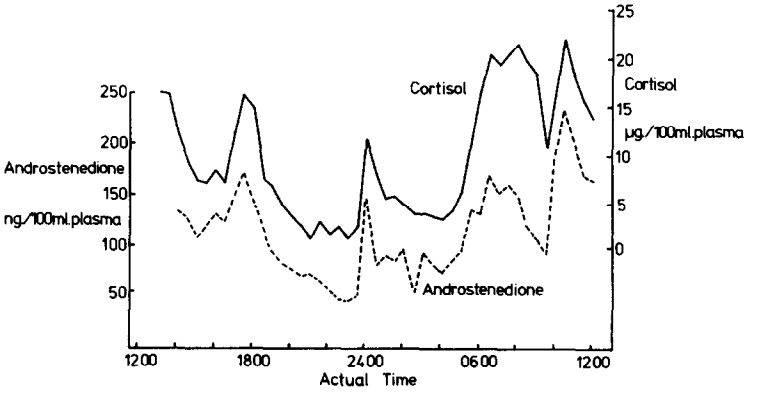

Fig. 8. Plasma cortisol and androstenedione levels through a 24-hour period in a normal subject.

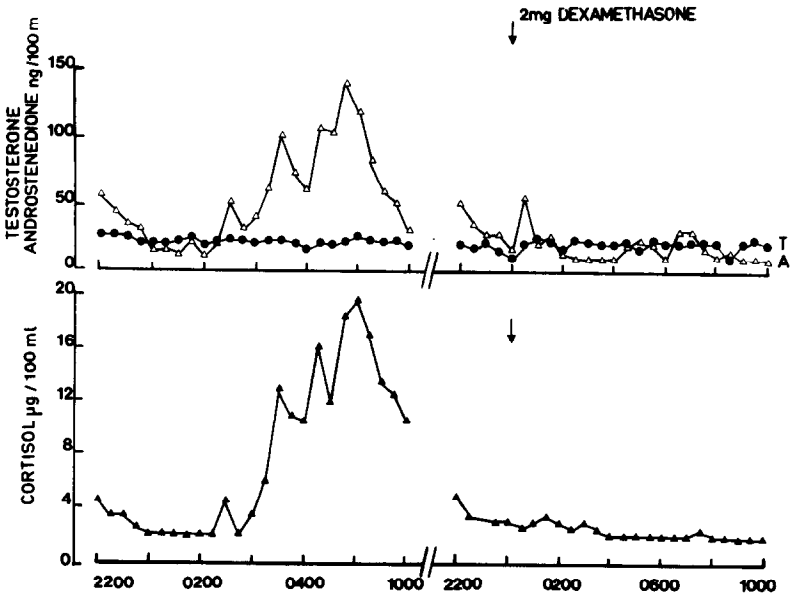

Fig. 9. Plasma steroid levels in an ovariectomised woman. 2 mg of dexamethasone was given orally at 24 hours on the second evening of the study.

suppression of pituitary ACTH secretion was achieved, and controlled ACTH stimulation could be carried out. This is done by constant infusion of physiological amounts of ACTH, using a carefully calibrated infusion pump.

Figure 11 illustrates the results from a typical study. In the control period, there is the usual correspondence between cortisol and androstenedione levels, which follow a characteristic episodic pattern. Testosterone levels are low and relatively stable. The oestrone and oestradiol levels are typically those seen in postmenopausal subjects, and oestrone also shows an episodic pattern, although one which is not obviously related to androstenedione. The effect of dexamethasone is to suppress the levels of all the steroids measured, which approach the limit of detection, in agreement with the assumption that their source in this patient is solely adrenocortical. Infusion of ACTH produces a rapid increase in cortisol levels, closely paralleled initially by androstenedione. Oestrone and oestradiol levels increase, but do so rather sluggishly compared with the other two steroids. In contrast to cortisol, the plasma levels of androstenedione do not achieve a steady plateau, but show considerable variation.

A number of points emerge from this study. First, it is apparent that plasma oestrone and oestradiol levels also tend to fluctuate, and indeed plasma levels of all the steroid hormones investigated show some degree of variation. The adrenocortical origin of the oestrogens is clear, since suppression of ACTH causes the virtual disappearance of these steroids from the plasma. ACTH in physiological amounts increases the plasma level of all the steroids, although only minimal changes were seen in oestradiol and testosterone. However, stimulation with ACTH restored cortisol, androstenedione and oestrone levels to approximately their presuppression values, thus illustrating the importance of ACTH in maintaining, presumably indirectly, circulating oestrone levels. Finally, the

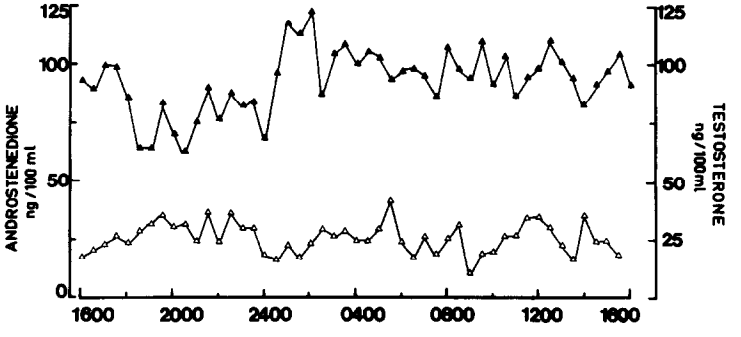

Fig. 10. Plasma androstenedione ▲——▲ and testosterone △——△ levels through a 24-hour period in a patient with Addison's disease.

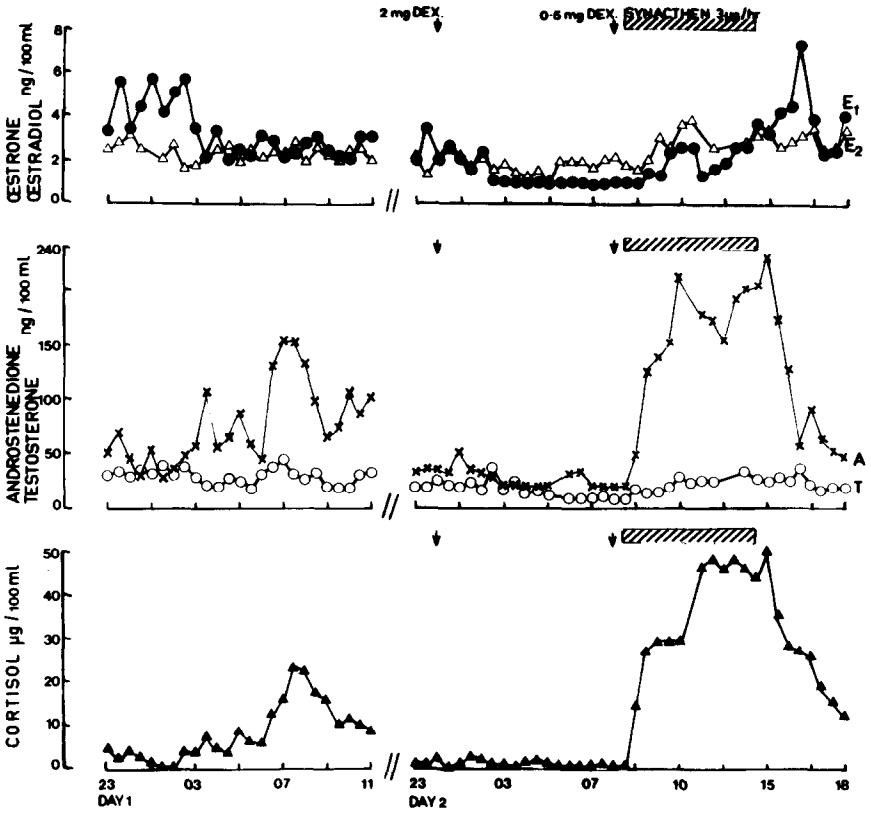

Fig. 11. Plasma steroid levels in an ovariectomised subject. Dexamethasone was given at 24 hours on the second evening, and an ACTH (Synacthen) infusion was commenced at 0830 hours.

marked variability of androstenedione levels during ACTH infusion suggests that either androstenedione secretion is episodic even when the adrenal cortex is stimulated continuously by ACTH, or that androstenedione metabolism is different from that of cortisol.

The interpretation of the results of these investigations is necessarily complicated by the fact that we have no direct means of discovering how the adrenal cortex responds to ACTH stimulation, and thus whether the variability which we see in androstenedione levels reflects pulsatile secretion, or if some other phenomenon (e.g. variable clearance rate) is involved. This problem is overcome by designing the experimental procedure in such a way that androstenedione entry into the vascular system can be rigidly controlled. In this way, it is possible to study the pattern of peripheral steroid levels under conditions of steady-state production. To achieve this, studies were made in ovariectomised patients, given suppressive doses of dexamethasone for a period of 7 days to deprive the adrenal cortex of ACTH stimulation.

Figure 12 illustrates the result of one such study. It shows that there is the usual pattern of steroid changes on the control day, and then following administration of dexamethasone, cortisol and androstenedione levels approach the limit of detection, confirming that steroid secretion must be absolutely minmal under these conditions. Whilst continuing the administration of dexamethasone, an infusion was commenced of 250 micrograms per hour of androstenedione, an amount intended to simulate that produced by the adrenal by stimulation with ACTH. This study then, is intended to mimic the situation in which ACTH was infused, but with complete control over steroid entry to the vascular system. By using a good quality infusion pump which had been previously checked for consistency of infusion rate, a steady rate of steroid entry could be achieved.

The figure shows that immediately the infusion started, androstenedione levels increased rapidly, and oestrone and testosterone levels also increased. Since in this study, the only steroid introduced was androstenedione, the results show quite clearly that rapid and significant conversion of androstenedione to both oestrone and testosterone occurs, levels of which approximate to those seen before suppression. The data also reveal that even though androstenedione is being infused at a steady state, the plasma levels, both of this steroid, and its conversion product oestrone, fluctuate markedly. It is notable too, that there is no clear relationship between plasma androstenedione levels and oestrone.

It might be argued that the fluctuating steroid levels seen are due to malfunction of the infusion

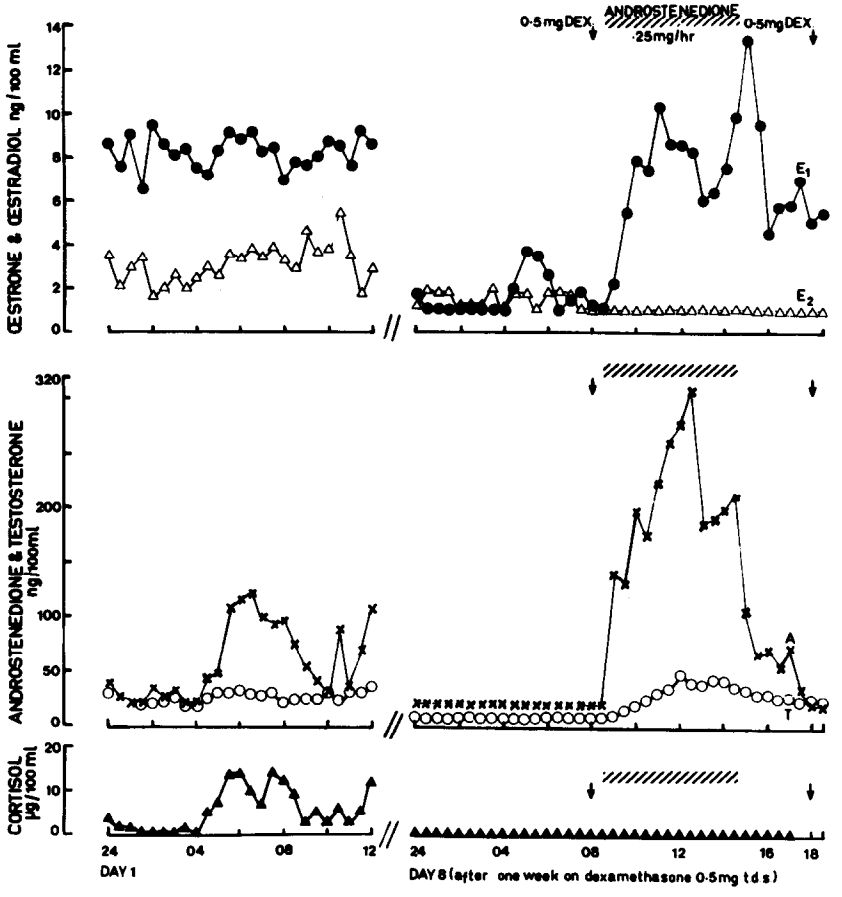

Fig. 12. Plasma steroid levels in an ovariectomised subject. Dexamethasone was given for one week, and androstenedione was infused as shown.

pump. If so, this would have to be very gross. However, it seems unlikely that this was so, since in a separate study, by infusing simultaneously another steroid, cortisol, whose dynamics are better known, it was found that this steroid reaches the expected plateau. The variability of the androstenedione levels contrast markedly with the smooth plateau achieved for cortisol.

The study illustrates clearly that androstenedione metabolism differs from that of cortisol. An explanation for the rapid fluctuations seen in plasma levels could be that the metabolic clearance rate of androstenedione changes rapidly. Caution is desirable therefore in interpreting fluctuating steroid levels as always indicative of episodic secretion.

The changes in oestrone levels are also complex and difficult to explain. Variations in the rate of conversion of androstenedione to oestrone, and alterations in metabolic clearance may both be significant factors, together with conversion to and from oestrone sulphate.

There are a number of conclusions to be drawn from these studies, which were originated largely to explore in more detail the nature of basal steroid secretion, in the hope of clarifying the mechanisms controlling steroid hormone levels. It seems clear from these, and many studies by other investigators, that the characteristic feature of basal plasma steroid levels is not that they approach a steady-state condition, but on the contrary, fluctuate markedly. It has been well demonstrated [1] that for cortisol at least, this represents episodic secretion, and it would be surprising if this were not so for the other steroids which are ACTH dependent. Thus, both DHA and androstenedione would seem to be secreted synchronously with cortisol in response to the pulsatile secretion of ACTH. The mechanism by which aldosterone is secreted synchronously with cortisol requires further exploration. Improvement in methodology for measuring plasma renin activity or angiotensin may reveal a closer direct relationship with plasma aldosterone levels, although it seems that there is still need at present to postulate the existence of some as yet undefined alternative mechanism.

The fluctuations seen in androstenedione levels appear to be largely explicable as ACTH-driven; nevertheless, androstenedione physiology is clearly somewhat complex, since the data reported here for androstenedione levels after infusion of this steroid, or after stimulation with physiological amounts of

ACTH, suggest that the clearance of androstenedione, unlike cortisol, undergoes rapid change, as indeed does the conversion of androstenedione, and other precursors, into oestrone.

Lastly, these data again underline the central and important role of the ACTH-adrenal axis in the production of several steroid hormones. In the postmenopausal female in particular, androstenedione, testosterone and oestrone secretion are all markedly ACTH-dependent and any description of steroid endocrine physiology or pathology will need to take account of this.

REFERENCES

1. Hellman L., Fujinori N., Curti J., Weitzman E. D., Kream J., Roffwarg H., Ellman S., Fukushima D. K. and Gallagher T. F.: Cortisol is secreted episodically by normal man. *J. clin. Endocr. Metab* **30** (1970) 411–422.
2. Krieger D. T., Allen W., Rizzo F. and Krieger H. P.: Characterization of the normal temporal pattern of plasma corticosteroid levels. *J. clin. Endocr. Metab.* **32** (1971) 266–284.
3. Yates F. E. and Urguhard J.: Control of plasma concentrations of adrenocortical hormones. *Physiol. Rev.* **42** (1962) 359–443.
4. Krieger D. T. and Gewirtz G. P.: The nature of the circadian periodicity and suppressibility of immunoreactive ACTH levels in Addison's disease. *J. clin. Endocr. Metab.* **39** (1974) 46–52.
5. Stockigt J. R.: Mineralocorticoid hormones. *Adv. Steroid Biochem. Pharmacol.* **5** (1976) 161–222.
6. Tunbridge D., Wilson G. A., Short F. and James V. H. T.: Control of eposodic secretion of aldosterone in man. *J. Endocr.* **65** (1975) 25p.
7. James V. H. T., Tunbridge R. D. G. and Wilson G. A.: studies on the control of aldosterone secretion in man. *J. steroid Biochem.* **7** (1976) 941–947.
8. Rosenfeld R. S., Rosenburg B. J., Fukushima D. K. and Hellman L.: 24-Hour secretory pattern of dehydroisoandrosterone and dehydroisoandrosterone sulfate. *J. clin. Endocr. Metab.* **40** (1975) 850–855.
9. Tunbridge D., Rippon A. E. and James V. H. T.: Circadian relationships of plasma androgen and cortisol levels. *J. Endocr.* **59** (1973) XX1.
10. James V. H. T., Rippon A. E. and Jacobs H. S.: Plasma androgens in patients with hirsutism. In *The Endocrine Function of the Human Ovary.* (Edited by V. H. T. James, M. Serio and G. Giusti) Academic Press, London (1976) p. 457.