SESSION 2

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Hirsutism and anovulatory infertility

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ANOVLATORY infertility is sometimes associated with an abnormal degree of hirsutism and, very occasionally, with more serious manifestations of virilization. In such cases it is usually possible to demonstrate excessive concentrations of androgenic steroids but these steroids may be derived from either the ovary or the adrenal cortex or from both these sources. This subject has been reviewed recently by Ferriman (1969), Ismail & Loraine (1969) and Prunty (1967).

Androgens and hair growth

The body surface of the infant, with the exception of the scalp and eyebrows, is covered with fine, soft, light coloured hair; this is the vellus hair. Later in life, the hairs at certain specific sites may become replaced with longer, coarser and darker terminal hair. This transformation of the vellus hair into the terminal hair appears to be governed by androgens. The amount of androgen necessary to effect this change depends on a number of factors. Among these factors are the race, sex and age of the individual and the site of the follicle on the body. Thus the transformation of pubic and axillary hair takes place under the influence of the small amount of androgen produced by the normal female at the time of puberty. Larger amounts of androgen are required for the transformation of hair follicles on the back or abdomen, for example. Since all the hair follicles of any one person are presumably subjected to the same androgenic stimulus, they must have different threshold levels of response.

There are clearly racial differences in the distribution of hair. Facial hair is more common in Mediterranean women than in other European women and very much more common than in Eastern women. No racial differences in parameters of androgenic steroids have been found although they have been sought (Mansuwan & Kalant, 1965).

Therefore, here also there are presumably different threshold levels of response to androgens. There are also threshold variations with age and, as with any other biologically graded characteristic, variations in sensitivity between normal individuals.

It now seems possible that even these differences in end-organ sensitivity may have a basis in steroid biochemistry. It has recently been reported (Wilson & Walker, 1969) that skin from certain sites, notably clitoris, scrotum and labia majora, has a much greater ability to convert testosterone into dihydrotestosterone (17β -hydroxy-5 α -androstan-3-one) than skin from other sites, e.g. skin from the limbs. Dihydrotestosterone is an even more potent androgen than testosterone and it seems possible that the ability of the tissue to form this dihydro-metabolite may be related in some way to the capacity to respond to testosterone.

Interrelations of androgens in the female

Although testosterone is the characteristic male hormone secreted by the testes, small amounts of this hormone are also secreted by the ovary and the adrenal cortex of the female (Fig. 1). However, testosterone is not the only androgenic hormone we must consider in the female. A number of other weaker androgens are also secreted: androstenedione from both ovary and adrenal cortex; dehydroepiandrosterone and its sulphate ester, both from the adrenal cortex. These are less androgenic than testosterone but, because they are secreted in much larger quantities, they may contribute significantly to the overall androgenicity. The androgenicity of these weaker androgens may be due largely, if not entirely, to their conversion to testosterone after secretion. The rate of secretion of androstenedione in the normal woman is about twenty times that of testosterone so that although only a small proportion of the androstenedione is

converted to testosterone, about 50% of the testosterone in female blood has been converted from androstenedione.

The metabolites of all these steroids are excreted in the urine mainly as 17-oxosteroids (17-OS). Some of the dehydroepiandrosterone sulphate is excreted unchanged but some is metabolized via androstenedione to the 11-deoxy 17-OS, androstosterone and aetiocholanolone (Fig. 1). Most of the androstenedione and testosterone is also metabolized by this route but a small amount of the testosterone, about 1%, is excreted as the glucuronic acid conjugate of testosterone itself. 11-Hydroxyandrostenedione has negligible androgenic activity but this too is excreted largely as 17-OS. Cortisol has also been included in the secretions of the adrenal cortex shown in Fig. 1. This is because a small proportion of this hormone (about 5%) is excreted as 17-oxosteroids although of course the major portion is eliminated as 17-oxogenic steroids (17-OGS).

The urinary 17-OS are thus a very complex group being derived from both adrenal cortex and ovary and from androgens of high and low biological activity and indeed, from steroids which are not androgens at all. It is possible therefore, that if there is excessive secretion of the potent androgen testosterone, the 17-oxosteroids may be within the normal range.

Excess production of androgen can originate from either the ovary or the adrenal cortex. The secretion of androgens by the ovary is not perhaps surprising if one examines the pathways by which oestrogens are synthesized (Fig. 2). There are two main pathways; the upper one appears to predominate in the follicle and the lower one in the corpus luteum (Ryan & Smith, 1965). Whichever of these pathways is followed, synthesis of the oestrogens,

oestrone and oestradiol can only proceed via the androgens, androstenedione and testosterone. The possibility of excess androgen production from the ovary is clearly present.

Hirsutism of adrenocortical origin

The most common type of hirsutism of adrenocortical origin is *simple or idiopathic hirsutism*. The excretion of 17-oxosteroids is usually high or in the upper range of normal. Treatment with prednisone or some other potent glucocorticoid results in suppression of the 17-oxosteroids to low levels indicating that the high pretreatment excretion of 17-oxosteroids is of adrenocortical origin. Since these patients usually have normal menstrual function they do not come within the scope of this review and they will not be considered any further.

Some cases of *Cushing's syndrome* have hirsutism and amenorrhoea. Mild facial hirsutism is relatively common in this condition. Again the 17-oxosteroid excretion may be normal or high but suppression with dexamethasone may not be possible and, in any event a higher dose than normal will be necessary. As in the case of simple hirsutism, the excretion of pregnanetriol is normal but menstruation is irregular or absent.

A rare cause of virilization is an *adrenal tumour*. In most of these patients the excretion of 17-oxosteroids is greatly increased and cannot be suppressed with prednisone or dexamethasone. Very high excretions of the weak androgen dehydroepiandrosterone are often found but the excretion of pregnanetriol is normal. Menstruation is irregular or absent.

Severe virilization is also seen in *congenital adrenal hyperplasia*. This rare condition is due to an inborn error of adrenal metabolism such that the adrenal cortex is incapable of the normal

FIG. 1. This shows the principal C₁₉ steroids secreted, their glands of origin, interconversion and metabolic fate.

Fig. 2. The main pathways for the biosynthesis of the oestrogens. The upper (Δ^5) pathway (heavy arrows) predominates in follicular tissue and the lower (Δ^4) pathway in luteal tissue.

synthesis of cortisol. The condition is usually detected at birth but occasionally a similar syndrome becomes apparent only after puberty. The 17-oxosteroid excretion is usually high but can be readily suppressed with prednisone. Likewise the high excretion of pregnanetriol, which is characteristic of this type of adrenal hyperplasia, can also be suppressed.

In the normal subject the secretion of cortisol into the blood serves to restrict the secretion of corticotrophin releasing factor (CRF) and ACTH (Fig. 3). In congenital adrenal hyperplasia there is a failure of this feedback control because of a failure to synthesize cortisol. This failure of cortisol synthesis is only partial so that secretion of CRF and ACTH increases until adequate concentrations of cortisol are reached. There is no defect in the secretion of androgens so that very large amounts of these steroids may be secreted. Administration of a glucocorticoid will suppress the excessive secretion of CRF, ACTH and androgens and will halt the progress of the disease.

Within the rectangle of Fig. 3, representing the adrenal cortex, are shown simplified pathways for the biosynthesis of cortisol and the androgens. The cortisol and androgen pathways diverge from 17-OH progesterone, the desmolase giving androstenedione and the 21-hydroxylase giving 11-deoxy cortisol which is then hydroxylated at C-11 to give cortisol. Enzymatic blocks at positions a, b or c can cause virilization although in the post-pubertal form of the disease defects have been reported only at b or c. The most common site of enzyme block is at b, involving the 21-hydroxylase. The high levels of ACTH resulting from the lack of feedback control result in the accumulation of those intermediates which are situated earlier in the biosynthetic pathway than the metabolic block. There is therefore an accumulation of pregnenolone, 17OH-pregnenolone and especially 17OH-progesterone. This latter steroid is secreted into the adrenal vein blood in high concentration, subsequently metabolized to pregnanetriol and, in this form, excreted in the urine. The 21-hydroxylase block does not interfere with andro-

FIG. 3. This figure shows the position of those biosynthetic blocks in cortisol synthesis which have virilizing effects. The relationship between these metabolic blocks and the urinary steroids characteristic of each of these lesions is shown by the interrupted arrows.

gen synthesis so that this too proceeds at a greatly increased rate. There is an isolated report of a 21-hydroxylase deficiency which became manifest in a 24-year-old woman immediately following a successful full-term pregnancy (Faglia *et al.*, 1969).

The other metabolic block is in the last stage of cortisol synthesis, the 11-hydroxylation; 11-deoxy cortisol is the intermediate occurring immediately before the block. This steroid is found in the blood and its tetrahydro-reduced derivative is excreted in the urine. Again large amounts of androstenedione may be produced and excreted as 17-oxosteroids.

Hirsutism of ovarian origin

Ovarian tumours

Recently reviewed by Prunty (1967), these are a rare cause of virilization. Various types of ovarian tumours are found, depending on the cell types from which they are derived. The most common are the arrhenoblastomas but virilization is also found with hilar cell and adrenal rest tumours. Only in the adrenal rest tumours are the excretions of 17-oxosteroids usually significantly elevated. However, the concentration of testosterone in blood and of its

glucuronide in urine may be greatly increased in all three types. Granulosa-theca cell tumours usually secrete oestrogen rather than androgen, but isolated cases involving virilization have been reported.

Polycystic ovaries

Because of the histological abnormalities of the ovary, this gland was considered the prime suspect as the source of the excess androgen in this condition. There is now a considerable body of evidence supporting the view that excess androgen is secreted by the polycystic ovary:

Evidence for an abnormality of ovarian steroidogenesis

(1) Removal of some of the ovarian tissue in the operation of wedge resection has favourable effects in a proportion of patients. In one series, regular cycles occurred in 80% of 447 cases although a decrease of hirsutism was only noted in 16% of 205 cases (Goldzieher & Green, 1962). Wedge resection has also been shown to cause a fall in the rates of secretion of androstenedione and testosterone (Jeffcoate *et al.*, 1968a).

(2) Stimulation with follicle-stimulating hormone (FSH) preparations has given increased secretion of androgens (Jeffcoate *et al.*, 1968a).

(3) Suppression of the adrenal cortex with dexamethasone does not invariably result in marked suppression of androgen secretion. Mahesh & Greenblatt (1964) found that some patients with polycystic ovaries showed poor suppression of 11-deoxy 17-oxosteroid after administration of glucocorticoid. However, administration of stilboestrol to these subjects caused marked suppression, suggesting that the 11-deoxy 17-oxosteroids were of ovarian origin.

(4) The high concentration of androstenedione in cyst fluid. The concentration of oestrogens however, is quite low so that the ratio of androstenedione: oestrogen may be one hundred times greater than that found in normal cyst fluid (Short, 1965).

(5) Biosynthetic studies with polycystic ovarian tissue indicate a similar conclusion, i.e. a rate of synthesis of androgens which is high relative to the synthesis of oestrogens (Jeffcoate *et al.*, 1968b). These studies have been made by incubating slices of the ovarian tissue removed at wedge resection with precursors of the steroid hormones labelled with radio-active isotopes.

(6) The best way of establishing the hormonal output of an endocrine gland is, of course, the analysis of the blood actually leaving the gland. Unfortunately specimens of ovarian vein blood are difficult to obtain and furthermore the ovarian veins do not always drain only the ovary. Nevertheless such studies as have been made have mostly indicated a greatly increased concentration of androstenedione in the ovarian vein blood but a normal concentration of oestradiol. This abnormality in the pattern of ovarian secretion is much reduced if the patients are treated with FSH before collection of the ovarian vein blood. This effect of treatment with FSH normalizing the steroid pattern of the polycystic ovary is shown also by other parameters, e.g. the ratio of androstenedione production to oestrogen production and the composition of the steroid fraction of cyst fluid (Jeffcoate, *et al.*, 1968b).

There is therefore a considerable body of evidence that the polycystic ovary produces excess androgen; we must now consider if this is the only source of the excess androgen.

Evidence for an abnormality of adrenocortical steroidogenesis

(1) There is an association of polycystic ovaries with adrenal androgenizing conditions. For example there are some reports of polycystic changes in cases of virilizing adrenal adenoma. However, there is some doubt whether typical polycystic ovaries are found.

(2) The administration of corticosteroids to patients with polycystic ovaries sometimes results in an improvement in ovarian function, a decrease in the degree of hirsutism and in the secretion of androgens.

(3) Patients with polycystic ovaries often excrete increased amounts of 11-oxopregnanetriol. The presence of the oxygen function at C-11 indicates that this steroid is of adrenocortical origin.

(4) What could be perhaps the most important piece of evidence, the demonstration of increased concentrations of testosterone and androstenedione in the adrenal vein blood of patients with polycystic ovaries, has not yet been obtained. The concentration of testosterone in adrenal vein blood from normal women has been measured and found to be about ten times that in ovarian vein blood. A raised concentration of testosterone in adrenal vein blood has been reported in idiopathic hirsutism (Burger, Kent & Kellie, 1964).

Several groups of investigators have reported raised concentrations of testosterone in the peripheral plasma of patients with polycystic ovaries (Bardin & Lipsett, 1967; Horton & Neisler, 1968; Southren *et al.*, 1969). However, it seems likely that this increase in the plasma concentration of testosterone does not fully represent the extent of the increase of testosterone available to the tissues. Southren *et al.* (1969) have shown that there is a large decrease in the *in vitro* binding of testosterone to plasma protein in subjects with polycystic ovaries. It therefore seems likely that the biologically active portion of the plasma testosterone (non-protein bound) will be raised to an even greater degree than the total plasma testosterone. The increase in concentration of testosterone in plasma associated with polycystic ovaries is also an underestimate of the increase in the production of this hormone since there is an approximate doubling of its metabolic clearance rate (Bardin & Lipsett, 1967). It is concluded therefore, that the hirsutism in polycystic ovary syndrome is due to an increase in the glandular secretion of testosterone rather than to the conversion of secreted androstenedione.

Although there is undoubtedly evidence for increased secretion of both androstenedione and testosterone by the polycystic ovary, the major source of the increased secretion of testosterone in this condition appears most often to be the adrenal cortex. Thus, Bardin, Hembree & Lipsett (1968) found that acute suppression of the adrenal cortex with dexamethasone caused a reduction of the concentration of testosterone in the plasma of more than 50% in four cases out of eight. The actual contribution of the adrenal cortex to the concentration of testosterone in the plasma is probably even greater than these figures suggest. Suppression of

the adrenal cortex of normal women with dexamethasone results in a decrease in the plasma concentration of testosterone of only about 25%, whereas measurements of the ovarian venous effluent suggests that the ovary contributes less than 20% of the secreted testosterone (Horton, Romanoff & Walker, 1966). A considerable portion of the testosterone secreted by the adrenal therefore appears to be, at least in the short term, independent of ACTH. Horton & Neisler (1968) administered prednisone to five women with polycystic ovaries for 1 month and found that they all showed a decrease of at least 50% in the concentration of testosterone in the plasma.

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