SUMMARY OF BIOCHEMICAL, MOLECULAR GENETIC and CLINICAL FINDINGS IN STEROID-RELATED DISORDERS

Introduction

Steroids in serum and urine comprise a large number of compounds of similar structure, providing an analytical challenge which has been met by use of multicomponent methods based on high resolution chromatography and mass spectrometry. Urinary steroid profiling by GC-MS has been instrumental in the identification of all the newly recognised inborn errors of steroid metabolism in recent decades. Panelling of hormonal steroids and their pathway intermediates in serum and saliva by LC-MS/MS promises to revolutionise clinical investigation. Until now, immunoassay has been the mainstay of this, but cross reaction by steroids in unusual excess can severely compromise specificity; further, immunoassays are not readily available for many useful markers.

The clinical effects of steroid–related disorders arise from deficiency or excess of the actions of hormonal steroids. For example, aldosterone functions to prevent sodium loss into the urine. Deficient aldosterone synthesis results in inability to retain sodium (salt wasting) while excess results in sodium and water retention and hypertension. Defects in the pathway of steroid response may mimic a deficiency. These can be due to defective steroid receptors or further downstream. For example, aldosterone deficiency is mimicked by mutations affecting the mineralocorticoid receptor or the protein that forms the sodium channel in the distal convoluted tubule. Excess production of steroid metabolic intermediates may result, paradoxically, from a steroidogenic defect, via loss of feedback inhibition. A defect in the last step to aldosterone results in excess corticosterone production via stimulation of the renin-angiotensin system. Excess hormonal steroid production can arise either via abnormal secretion of trophic hormone by a tumour or due to some other derangement of control or directly from tumours of the adrenals or gonads. Examples for aldosterone production are increase of renin from a reninoma, increase of renin as a result of renal artery stenosis and excess secretion of aldosterone from a Conn's adenoma in the adrenal cortex. In the following sections, findings in blood and urine are summarised before discussion of each disorder.

Defects of cortisol synthesis/action (congenital adrenal hyperplasia)

Cortisol secretion is controlled by the hypothalamo-pituitary-adrenal (HPA) axis. Cortisol feeds back to inhibit hypothalamic corticotrophin releasing hormone (CRH) secretion and pituitary adrenocorticotrophic hormone (ACTH) secretion. Thus, a lack of feedback as a result of diminished cortisol

concentration or defective cortisol response results in increases of CRH and ACTH and thus increase of adrenal activity. ACTH stimulates both adrenal steroid synthesis and growth, so chronic ACTH excess results in bilateral adrenal enlargement. For inborn conditions, this develops *in utero*, since the fetus has a functioning HPA axis, giving rise to the term *congenital adrenal hyperplasia*, (CAH). 'CAH' is often used as shorthand for *21-hydroxylase deficiency* because this is the commonest form of the disorder, representing some 95% of all steroid metabolic defects identified. It should be used to denote any of the 5 defects in the pathway of conversion of cholesterol to cortisol. A further 4 causes of adrenocortical hyperplasia can now be distinguished, so these are listed below and can also be considered to be forms of CAH.

Cholesterol to pregnenolone conversion defects: lipoid adrenal hyperplasia due to 20,22-lyase (*CYP11A1*) defect or StAR protein defect (*STARD1*)

Absence of all steroids in blood and urine.

Both adrenal glands and gonads are affected. The adrenal glands are characteristically enlarged and filled with lipid droplets. It was long assumed that the primary defect was in side chain cleavage of cholesterol by 20,22 lyase, but it is now clear that these are in the minority and in more cases there is a defect in StAR (Steroid Acute Regulatory) protein, which is synthesised in response to ACTH stimulation and is utilised for transporting cholesterol into the mitochondrion. Absence of mineralocorticoid results in salt wasting, of glucocorticoid results in hypoglycaemia and of sex steroids results in genetic males being phenotypically female. Consequences for secondary sexual development are unreported, since diagnosis and treatment take place early in life.

3β-Hydroxysteroid dehydrogenase (*HSD3B2*) deficiency

Increased serum 17α-hydroxypregnenolone and DHA and metabolites in urine. Low/absent androgen, corticosterone and cortisol in serum and metabolites in urine.
Affected patients on glucocorticoid treatment paradoxically excrete more 17-hydroxyprogesterone than 17-hydroxypregnenolone metabolites, presumably due to a relatively enhanced effect of peripheral 3βHSD 1 on a diminished amount of precursor.

In newborns, high levels of urinary pregnenolone and DHA metabolites together with low levels of cortisol metabolites are suggestive of the disorder. A similar pattern is also found in preterm, otherwise normal, infants. The disorder is confirmed biochemically if the pregnenolone and DHA metabolites

persist beyond 3 months post term, when they would normally have declined; pregnenetriol emerges as the steroid in greatest amount. After puberty, DHA becomes more prominent.

This defect affects steroid production in both gonads and adrenals. When severe, the production of all steroid hormones is nearly abolished. Deficiency of testosterone production in the male results in incomplete masculinisation of the external genitalia of the newborn (male pseudohermaphroditism, XY DSD); paradoxically, the female newborn has virilised external genitalia (female pseudohermaphroditism, XX DSD) as a result of excess of the mild androgen, DHA. The androgenic action of DHA may arise from conversion to testosterone in low yield by peripheral 3βHSD 1 rather than via binding to the androgen receptor. Deficiency of cortisol results in hypoglycaemia and of aldosterone in salt wasting.

17α-Hydroxylase (*CYP17A1*) deficiency

Increased serum progesterone, 11-deoxycorticosterone (DOC) and corticosterone and urinary metabolites. Absent androgens and cortisol in serum and their metabolites in urine; in the newborn, additionally, high levels of urinary pregnenolone metabolites and absent DHA metabolites.

17α-Hydroxylase combines both hydroxylase and 17,20 lyase activities. By site-directed mutagenesis, some separation of function within the protein has been recognised. Since 17α-hydroxylation precedes side chain cleavage, the biochemical consequences of a 17α-hydroxylase deficiency are the same whether side chain cleavage is impaired or not. No conversion of C21 to C19 steroids is possible, resulting in abolition of production of androgens and oestrogens. Cortisol and cortisol precursors are also absent. Genetic males are phenotypically female. Neither gender develops secondary sexual characteristics. Deficiency of cortisol production does not result in glucocorticoid deficiency, because corticosterone, a mild glucocorticoid, substitutes. Stimulation of this remaining pathway results in excess of DOC, which has mineralocorticoid activity and so leads to hypertension.

Mutations of *CYP17A1* affecting only lyase activity have been claimed in the literature. However, all reported patients who were subjected to a synacthen stimulation test showed an impaired cortisol response, indicating that there is also diminished 17-hydroxylase activity. Specific lyase defects due to cytochrome B5 gene mutations have recently been described (see later).

21-Hydroxylase (*CYP21A2*) deficiency

Increased serum 17-hydroxyprogesterone, 21-deoxycortisol and androgens and urine metabolites. In the severe (*classic*) form, cortisol production is

very low but immunoassays almost always produce normal to high serum levels due to cross reaction with excess cortisol precursors. Cortisol metabolites are detectable in urine but at very low levels. They are paradoxically increased above normal in 24h collections from patients with a partial (*non classic*) defect: this may be due to excess 21-deoxycortisol competing with cortisol for the glucocorticoid receptor, thus attenuating negative feedback. In the newborn, numerous additional metabolites of 17α-hydroxyprogesterone and 21-deoxycortisol are present, several of which are better diagnostic markers than the classical markers that predominate later.

Increased androgen levels result in virilisation of the external genitalia in newborn girls. Two presentations of the classic form are distinguishable: the *simple virilising* and the *salt wasting* forms. No biochemical distinction is possible, but they must differ in the ability to make aldosterone. Since the normal production rate of this steroid is very low in comparison to that of cortisol, small differences in 21-hydroxylase activity close to zero may be crucial. In boys, the defect is missed at birth unless newborn screening is in use. If they have the salt wasting form, they present with dehydration etc. at around 18 days of life. Boys with the simple virilising form are first found with early appearance of pubic hair and penile enlargement at 2-4y of age. If they are not treated, the continuing androgen excess leads to sexual precocity and increased growth, but they have short final height as a result of epiphyseal closure. Those with the simple virilising form may also show high renin levels and so benefit from mineralocorticoid supplementation. Undertreated males may develop benign testicular masses.

Partial deficiency usually presents with symptoms of androgen excess before puberty. Urinary steroid profiling enables unequivocal detection of a clinically significant enzyme deficit without the need for synacthen stimulation.

11β-Hydroxylase (*CYP11B1*) deficiency

Increased serum DOC, 11-deoxycortisol, 17-hydroxyprogesterone and androgens and urinary metabolites. Very low serum cortisol and urinary metabolites. Immunoassay of 11-deoxycortisol is especially unreliable in this disorder. Cortisol immunoassay may show normal to high levels due to cross reaction with precursors. A similar metabolome is found when patients are on treatment with drugs which inhibit 11β-hydroxylase activity, including metyrapone and azole antifungals. Increased 11-deoxycortisol is also found in association with some adrenocortical carcinomas.

Increased androgen levels result in virilisation of the external genitalia in newborn girls. Increased production of DOC, a mild mineralocorticoid, results in hypertension. In boys, the defect may be missed at birth but be signalled later by early appearance of pubic hair and penile enlargement. If untreated,

4

the androgen excess results in sexual precocity and increased growth, but short final height as a result of epiphyseal closure.

Apparent cortisone reductase deficiency and cortisone reductase (*HSD11B1*) deficiency

These are due to deficiency of hexose-6-phosphate dehydrogenase (*H6PD*) and 11-hydroxysteroid dehydrogenase I respectively. Serum steroid levels, including of cortisol, are *normal.* In urine there is a decreased ratio of cortisol/cortisone metabolites and an overall increase of adrenal steroid metabolites. Deficiency of hexose-6-phosphate dehydrogenase produces this metabolome because it is essential for activity of 11-hydroxysteroid dehydrogenase I, being required to regenerate NADPH, its hydrogen donor, in the endoplasmic reticulum.

The 11-oxoreductase defect results in an approximately tenfold increase in cortisol clearance rate. Cortisol levels in the blood are maintained by chronically increased adrenal stimulation. As a consequence, adrenal androgen production is also increased, resulting in hirsutism in both men and women and menstrual irregularity and infertility in women. Menstrual cyclicity is readily restored by low dose dexamethasone treatment, which emphasises the deleterious effects of adrenal androgen excess.

Cytochrome P450 oxidoreductase (*POR*) deficiency (associated with Antley-Bixler syndrome)

Increased corticosterone and 17-hydroxyprogesterone in serum and of the corresponding metabolites in urine. In the newborn, relative lack of steroids with additional hydroxyl groups. In a pregnancy with an affected fetus, low maternal oestriol, high ratios of urinary 5α/5β-reduced steroids, especially androsterone/aetiocholanolone and of the 5α/5β epimers of 17-hydroxypregnanolone.

Cytochrome P450 oxidoreductase has a role in supplying electrons to the steroid hydroxylases, which are cytochrome P450 enzymes. Deficiency may thus impair any of these enzymes but particularly affects 17-hydroxylase and 21-hydroxylase, producing a biochemical profile that is a mixture of the two disorders. However, relative enzyme deficits are variable, presumably because different mutations differentially affect interaction of POR with the various cytochrome P450s. The male at birth shows incomplete masculinisation of the external genitalia as a result of deficient androgen production, while paradoxically, the female newborn has virilised external genitalia, which do not further virilise after birth. This has been hypothesised to be the result of androgen synthesis by an alternative ('backdoor') pathway via the 5α-epimer of 17-hydroxypregnanolone which is only important in the

fetus. There are cranial and other bone malformations, which may be due to cholesterol deficiency and accumulation of toxic sterol intermediates.

7-Dehydrocholesterol reductase (*DHCR7*) deficiency, (associated with Smith Lemli Opitz Syndrome, SLOS)

This enzyme catalyses the last step in the cholesterol synthesis pathway. Increase of serum 7-dehydrocholesterol. This is metabolised to 7- and 8-dehydro forms of the usual steroids in both pregnancy and the newborn, with 7-dehydro oestriol a useful marker in urine from pregnant women. Clinical problems include mental disability and dysmorphia, including syndactyly, probably resulting from cholesterol deficiency and accumulation of toxic sterol intermediates.

Glucocorticoid receptor (*NR3C1*) defects

Serum cortisol is elevated but shows normal circadian variation. Urine cortisol, corticosterone and adrenal androgen metabolite levels are elevated.

As with cortisone reductase defect, the adrenals are chronically hyperstimulated, leading to androgen overproduction and hirsutism in women and to DOC overproduction, resulting in hypertension. Since cortisol feedback control operates via the glucocorticoid receptor, a decreased affinity for cortisol results in the concentration of cortisol in the blood being automatically appropriately increased.

Defects of sex hormone synthesis/action

Listed below are those not already described as forms of CAH (see section above). They result in primary or secondary effects on sexual differentiation, now collectively classified as disorders of sexual differentiation (DSDs).

Apparent 17,20-lyase (*CYP17A1*) deficiency

Although isolated impairment of 17,20-lyase activity due to *CYP17A* mutations has been claimed in the literature, affected individuals all have an impaired cortisol response to synacthen, showing that 17-hydroxylation is also compromised. A metabolome of defective production of androgens and oestrogens but not cortisol and also increase of 17-hydroxyprogesterone metabolites has been found in association with cytochrome B5 gene (*CYB5A*) mutations. This cytochrome was formerly regarded as a permissive factor, but is clearly essential, providing electrons to *CYP17A*.

17β-Hydroxysteroid dehydrogenase (*HSD17B3*) deficiency

Increased serum ratio of androstenedione/testosterone. In prepubertal subjects, HCG stimulation is required to show the abnormality. No definitive changes are seen in urine metabolites, although an increase of androsterone/aetiocholanolone has been observed in one kindred, and these androstenedione metabolites may be increased post puberty as a result of failure of feedback inhibition of LH by testosterone.

The Type 1 enzyme (*HSD17B1*) is required for conversion of oestrone to oestradiol, and thus deficiency might have similar effects to 17α-hydroxylase deficiency on sexual differentiation in females.

5α-Reductase 2 (*SRD5A2*) deficiency

Increased serum ratio of testosterone/dihydrotestosterone. In prepubertal subjects, HCG stimulation is required to show the abnormality. Increased ratio of 5β/5α reduced urinary metabolites of androgens, corticosterone and cortisol metabolites, with cortisol metabolites the most diagnostic (the *tetrahydocortisols*).

In healthy newborns, none of the 5β/5α reduced pairs of urinary steroid metabolites are detectable at birth. Tetrahydrocortisols can be distinguished by high sensitivity selected ion monitoring GC-MS by about 20 days post full term. Unfortunately, in 5α-reductase 2 deficiency this increase is delayed and it may be up to 3 months before a clear diagnosis can be made. Thereafter, urinary steroid profiling has proven to provide more reliably identification than plasma analysis. This may be because serum DHT partly arises by reduction by 5α-reductase 1, while production of 5α-tetrahydrocortisol depends solely on activity of 5α-reductase 2. If patients have already been gonadectomised, the serum testosterone/dihydrotestosterone ratio can no longer be utilised but urinary tetrahydrocortisols can still be used for diagnosis.

The existence of this defect in isolated communities in the Dominican Republic first pointed to the importance of dihydrotestosterone (DHT) as a potent androgen. Boys are born with incomplete masculinisation, but show significant virilisation at puberty, suggesting that DHT is more important for development of the external genitalia *in utero* but testosterone is more important later, when increases are driven by LH as a result of maturation of the hypothalamo-pituitary-gonadal (HPG) axis, perhaps enhanced by attenuation of androgen-mediated feedback inhibition.

3α-hydroxysteroid dehydrogenase (*AKR1C1-4*) deficiency

Male DSD due to mutations in genes for 3α-hydroxysteroid dehydrogenase

types 2 and 4 has recently been reported. One family had a steroid profile first interpreted as a 17,20-lyase deficiency. This has been taken as evidence for the importance of the 'back door' pathway of androgen synthesis, but does not appear to explain the very low levels of androgen synthesis shown by urine profiling, since the usual route (ie: the 'front door' pathway) does not require this activity.

Androgen receptor (*AR*) defect (testicular feminisation)

Serum and urine androgen levels are normal or elevated in the male.

Absence of androgen effect results in a genetic male being phenotypically female. Sexual hair does not develop. Since in the male, testosterone can be converted to oestrogen, when it is unopposed by androgen there is development of breasts and female body habitus.

Oestrogen receptor defect and aromatase (*CYP19A1*) deficiency

Steroid levels are normal, except for deficiency of oestrogens in aromatase deficiency.

Female fetuses are virilised *in utero*. In both genders, these disorders result in failure of epiphysial closure of the long bones, so that growth continues into adulthood, leading to tall stature. Females show primary amenorrhoea. This demonstrates that steroid-induced bone maturation, previously ascribed to androgens, in fact operates via conversion to oestrogens.

Anabolic steroid abuse

This suppresses gonadotrophin production and so results in testicular atrophy in the male, with consequent reduction of androgen production and decrease of sperm count. If steroids other than testosterone are being taken, the androstenedione metabolites will be low. Since testosterone is often taken as well, normal levels of androstenedione metabolites do not exclude the use of other anabolic steroids.

Specific targeting of urinary metabolites of synthetic androgenic-anabolic steroids by a specialist sports doping laboratory can identify abuse of these, while testosterone abuse is detected by increase of testosterone/epitestosterone in serum and by isotope ratio measurements, which differentiate endogenous and exogenous sources.

Precocious puberty/virilisation in children

Increased secretion of androgens by the adrenals may result in early growth

of pubic and axillary hair without breast development in girls and to sexual hair growth without testicular enlargement in boys. This may be due to partial virilising forms of CAH or, rarely, to an androgen-secreting tumour. A much more common cause is *premature adrenarche*, which is conceived as a premature increase in adrenal androgen synthesis due to early maturation of the zona reticularis of the adrenal cortex. Affected patients do not usually progress early to true puberty, but may show advance of height and bone age. True precocious puberty can occur as a result of early maturation of the HPG axis. This can be idiopathic or caused by certain brain lesions, such as a hamartoma.

Premature adrenarche and premature puberty both result in increased urinary androstenedione metabolites. Increase of DHA metabolites provides positive evidence for adrenarche but a lack of increase does not negate adrenarche, because the rate of conversion of DHA to androstenedione shows great inter-individual variability, presumably consequent on variable 3β-hydroxysteroid dehydrogenase activity.

Isolated growth of pubic hair in the first years of life also has an idiopathic presentation: there are no increases of serum androgens or urinary androgen metabolites and the hair tends to diminish with time. Although this has been described in the literature only for boys, leading to suggestions that it is initiated by the *post partum* androgen surge, it also occurs in girls, suggesting an alternative aetiology.

Hirsutism in women

Excess testosterone in serum is a common finding, but concentrations may be normal in association with low sex-hormone binding globulin, which results in increased free steroid. Serum testosterone values in women obtained by immunoassay are unreliable.

Partial forms of virilising CAH rarely occur. Polycystic ovary syndrome (PCOS) is very common in this group. The polycystic ovary secretes excess androgens, but there is also evidence in many patients for increased adrenal secretion of androgens and cortisol. This may be due to chronic adrenocortical hyperstimulation secondary to glucocorticoid resistance or to enhanced cortisol clearance due to a relative increase of cortisol 11-dehydrogenation. Precocious adrenarche (above) may have similar origins: girls with precocious adrenarche tend to develop PCOS later.

Steroid sulphotransferase (*SULT2A1*) converts DHA to DHA sulphate and deficiency has been postulated to result in DHA excess and corresponding androgenisation. This enzyme requires 3'-phosphoadenosine-5'-phosphosulphate (PAPS), generated by PAPS synthase (*PAPSS1 & 2*) and a

PAPSS2 defect in a virilised girl has been reported.

Defects of mineralocorticoid synthesis/action

Those listed below do not include the three salt-wasting forms of CAH (described above). A defect in aldosterone production or action results in excess stimulation of precursor production via the renin-angiotensin system.

Newborn infants with salt wasting not due to CAH invariably show rapid decrease of the urinary pregnenolone and DHA metabolites which are normally at high levels at this period. This appears to be secondary to a 'drive' to make aldosterone and is probably mediated by angiotensin 2, since this hormone has been shown to induce apoptosis in the fetal zone of the adrenal cortex in cultured fetal adrenal explants.

Aldosterone synthase (*CYP11B2*) deficiency (corticosterone methyl oxidase (CMO) defect)

Increased corticosterone and low or normal aldosterone levels in serum. Increased urinary corticosterone metabolites. A type I CMO defect (lack of 18 hydroxylation) can be distinguished from a type 2 defect (lack of 18 oxidation) by relative absence or relative excess respectively of 18-hydroxylated corticosterone metabolites.

Absence of aldosterone synthesis results in salt wasting in the newborn. A consequent increase of renin stimulates the production of DOC and corticosterone. The DOC substitutes to some extent for aldosterone. Beyond early childhood, the condition is ameliorated, probably because patients have an increased salt appetite, so can compensate for their increased salt loss.

Pseudohypoaldosteronism (PHA)

All forms of PHA that lead to salt wasting show increased corticosterone and aldosterone in serum and their metabolites in urine. *Type 1* PHA has two genetically determined forms, one of which is severe (autosomal recessive, more common, presenting in the first week) due to an apical sodium channel (*SCNN1B*) defect while the other is milder (autosomal dominant, less common, presenting later) due to a mineralocorticoid receptor (*NR3C2*) defect. *Type 2* PHA (Gordon's syndrome) causes hypertension and hyperkalaema *but not salt wasting*. It is due to defects of lysine deficient protein kinase (*WNK1 & WNK4*). *Secondary* PHA Type 1 (named *Type 3* by some authors) most commonly arises from urinary tract infection or urinary tract abnormality, with or without associated infection. A rarer cause is severe exudative eczema, in which electrolytes are lost in the exudate. There

is similarly increased aldosterone production, which represents an appropriate response to sodium loss though the kidneys or elsewhere. If PHA is due to urinary tract infection, cholesterol is increased in the urinary steroid profile. It probably originates in epithelial debris. Clinical consequences of PHA are similar to those of aldosterone synthase defect.

DAX-1 (*NROB1*) defect

Major deletions can result in salt wasting. The urinary steroid profile shows only cortisol metabolites, without the relative increases of corticosterone metabolites expected in the causes listed above. See also the section on congenital adrenal hypoplasia below.

Corticosteroid 11-dehydrogenase (*HSD11B2*) deficiency

Serum steroid levels, including of cortisol, are *normal.* In urine, there is an increased ratio of urinary cortisol/cortisone metabolites, of cortisol/cortisone and decreased adrenal steroid metabolites as a result of decreased cortisol clearance rate. The urine metabolite pattern invariably shows an increase of 5α- v. 5β-reduced tetrahydrocortisol.

This defect is also known as *apparent mineralocorticoid excess*. Mineralocorticoid receptors in the distal convoluted tubule of the kidney have similar affinity for cortisol and aldosterone. They are, however, protected from exposure to cortisol by effectively complete conversion to cortisone by 11-dehydrogenase, which is also localised in the tubule. Enzyme deficiency therefore exposes the receptors to high levels of cortisol, resulting in severe hypertension and hypokalaemia.

Other disorders related to altered steroid production

Steroid sulphatase (*STS*) deficiency (placental sulphatase deficiency)

This disorder may be detected by chance in pregnancy when oestriol as quantified, eg: in the *triple test* for Down's syndrome. It is by far the most common cause of a very low value. Oestriol is mostly formed in pregnancy from sulphated precursors that arise from the fetus. These are first desulphated and then further metabolised in the placenta. Steroid sulphate levels are greatly increased in maternal urine and oestriol is low in maternal serum and urine.

The defect causes *X-linked ichthyosis,* (the skin has fish-like scales), probably resulting from diminished epidermal shedding consequent on accumulation of cholesterol sulphate in the dermis. Affected individuals show

increased serum cholesterol sulphate. There are no differences in urine steroid metabolites.

Congenital adrenal hypoplasia

This may be due to a primary failure of adrenal development or be secondary to absence of ACTH stimulation due to either defective pituitary production of ACTH or to a defect of ACTH response. All adrenal steroids in serum and urine may be low or absent in the primary form while angiotensin-dependent aldosterone production is intact in the secondary form. Primary failure may be X-linked, due to a DAX-1 (Dosage sensitive sex reversal, also called NROB1, *NROB1*) defect, or autosomal recessive, due to SF-1 (*NR5A1*, steroidogenic factor 1) defect. ACTH resistance (which is autosomal recessive in the various forms) may be due to a defect of the ACTH receptor (*MC2R*, Melanocortin Type 2 or MC2-R) or to MRAP (*MRAP*, Melanocortin Receptor Accessory Protein) defect. Isolated ACTH resistance is also known as *FGD* (Familial Glucocorticoid Deficiency), while Allgrove syndrome comprises alacrimia, achalasia and adrenocortical insufficiency plus neurological disorders (*AAAS*, Triple A syndrome). *FGD Type 2* has been used to describe ACTH response defects downstream of the ACTH receptor, which include MRAP defects.

Deficiency of cortisol results in hypoglycemia and of aldosterone results in salt wasting.   Individuals with primary adrenal failure tend not to spontaneously enter puberty, suggesting that adrenal androgen production has a priming role, but other functions of the affected gene may be involved.

Addison's disease

This is due to progressive destruction of the adrenal cortex, most commonly by autoimmune processes, but also by tuberculosis. Adrenocortical steroid levels may be normal as a result of trophic hormone (angiotensin and ACTH) stimulation, but decline when destruction is at an advanced stage. Autoantibodies to CYP21 are common in the autoimmune form and may cause decrease of activity of this enzyme (under investigation).

Two multisystem disorders that include adrenocortical insufficiency are adrenoleucodystrophy, a disorder of peroxisomal fatty acid β-oxidation, caused by defect of a peroxisomal membrane transporter protein (ATPase binding cassette, *ABCD1*) and Wolman disease, a disorder of liposomal acid lipase (*LIPA*), which specifically causes adrenal calcification.

Cushing's syndrome

High levels of cortisol in blood and saliva and of cortisol metabolites in urine.

The normal circadian rhythmicity of cortisol is lost in blood and saliva. The pattern of urine metabolites is distinctive, with increase of 5β/5α reduced and of 11-hydroxy/11oxo metabolites, increase of cortisol/androgen metabolites and of free cortisol. The faster the increase of cortisol production, the more marked these changes are. In contrast, chronic, stable, cortisol hypersecretion is associated with a normal pattern.

Cushing's arises from excess cortisol exposure. If endogenous, cortisol levels are high in blood and urine, and the normal circadian rhythmicity of cortisol is lost. This may be *primary*, resulting from autonomous overproduction by an adrenal tumour or *secondary*, as a result of ACTH excess, or *tertiary* as a result of CRH excess. ACTH excess may arise from the pituitary, (referred to as Cushing's *disease*) or from a tumour elsewhere (referred to as *ectopic ACTH syndrome*). Another form, which may be regarded as secondary, is *food dependent Cushing's syndrome,* in which gastric inhibitory polypeptide (GIP) receptors are abnormally expressed in adrenocortical tissue. A post prandial increase of GIP thus stimulates cortisol secretion. Chronic hyperstimulation may also result in bilateral nodular adrenal hyperplasia. Receptors for other hormones (vasopressin, serotonin etc.) may be similarly abnormally expressed. Cushing's syndrome may alternatively be *iatrogenic*, arising from treatment with glucocorticoids (by inhaled, topical or oral routes). These may be prescribed, secretly self-administered or present (and usually undeclared) in 'herbal' medicine preparations. Use of prednisolone may confuse investigations because it cross-reacts 100% in cortisol immunoassays; distinction is possible by LC-MS/MS or urine steroid profiling. Most other synthetic glucocorticoids cannot be detected by urinary steroid profiling, but their use would be indicated by suppression of endogenous steroids.

Glucocorticoid remediable hyperaldosteronism (GRA)

Increase of serum and urinary aldosterone and 18-hydroxycortisol. Recovery of urinary 18-hydroxycortisol is poor on urinary steroid profiling but quantification by LC-MS/MS in both media is possible.

GRA is due to formation of a chimeric gene involving an unequal crossing over at meiosis in which the 5' regulatory region of the 11-hydroxylase gene, *CYP11B1*, is joined to the coding region of the aldosterone synthase gene, *CYP11B2* (with which it shares 90% sequence homology). This leads to ACTH-dependent expression in the zona fasiculata of the adrenal cortex, resulting in aberrant synthesis of aldosterone and 18-hydroxycortisol. Hypertension due to aldosterone excess is ameliorated by glucocorticoid treatment, since it diminishes ACTH secretion.

Steroid-producing tumours

Adrenocortical tumours usually produce very distinctive patterns of steroid increase. Most are sporadic, but genetically determined forms exist: Li-Fraumeni syndrome due to lack of a tumour suppressor, *tumour protein 53* (*TP53*) and McCune Albright syndrome causing multinodular hyperplasia, due to a mutated G-protein signalling molecule (*GNAS*), which prevents down-regulation of cyclic AMP signalling.

In adults, adenoma and carcinoma are nearly always distinguishable by urinary steroid profiling. Steroid-producing adenomas may generate excess cortisol, leading to increased urinary cortisol metabolites. Another common pattern is relative increase of 11-hydroxylated androstenedione metabolites. In carcinoma, findings are very heterogeneous. There may be increases of andostenedione or cortisol metabolites or, more commonly, the patterns resemble those of one or more partial enzyme deficiencies, in that there are high levels of urinary metabolites of intermediates in the cortisol or aldosterone pathways. Relative increases of unusual steroids, not readily ascribed to a particular enzyme deficiency, are also common. If steroid sulphates are among the steroids that are increased, there may also be large increases of serum and urine cortisol sulphate. Whether this is detected by cortisol assays will depend on the method used.

In children, distinction of steroid patterns in adenoma and carcinoma is not so well established, since histological distinction is less certain. There is a peak of incidence of virilising tumours between 18 months to 4 years with, most commonly, high levels of DHA and DHA metabolites and, less commonly, increase of 11-hydroxylated androstenedione metabolites.

Conn's adenoma describes an aldosterone-secreting tumour, which usually has a distinctive yellow appearance and different origin from other adrenocortical adenomas. Some have recently been shown to be associated with mutations of the potassium channel Kir 3.4 (coded by *AKCNJ5*). Conn's adenomas do not usually show distinctive urine steroid profiles, but may secrete other steroids, including cortisol and 18-hydroxycortisol. The classical picture of a single discrete mass is probably never true: histologically, zona glomerulosa hyperplasia and other foci are also seen.

Very rarely, adrenal tumours secrete oestrogens. We have seen too few of these to be able to classify their steroid profiles, but reports in the literature indicate that other steroids may be simultaneously secreted.

Gonadal tumours may secrete androgens, oestrogens, pregnenolone, progesterone or 17-hydroxyprogesterone. If the latter is secreted, urinary metabolites do not include those of 21-deoxycortisol, enabling distinction

from 21-hydroxylase deficiency and other adrenal causes of 17-hydroxyprogesterone excess. If serum testosterone is increased and the source is an adrenal tumour, there are invariably large increases of androstenedione, DHA and DHA metabolites in urine; if it is a gonadal tumour, there may be no increases of urinary androgen metabolites.

For reasons that are not clear, DHA sulphate in blood and urine tends to be poorly correlated across the spectrum of disorders in which it is increased. This does not appear to reflect shortcomings in the analytical methods. It may relate to variable rates of renal clearance, since the metabolic clearance rate of this steroid is especially slow.

Clinical effects of steroid-secreting tumours are those of excess of the steroid hormones produced. If cortisol is secreted, the contralateral adrenal gland will be atrophied as a result of ACTH suppression. Cortisol production from a growing tumour may not become clinically apparent as Cushing's until the production rate from the tumour exceeds the normal daily production rate of cortisol ie: until the capacity of the HPA axis to compensate by down regulation has been exhausted. Following surgery it is necessary to supplement with glucocorticoid in a diminishing dose to enable the remaining gland to recover. Clinical experience suggests that recovery of adrenal function is not always complete, and so lifelong steroid supplementation may be necessary. Occasionally, ACTH is not fully suppressed in primary Cushing's syndrome. This may be a result of competition for the glucocorticoid receptor by tumour steroid products, of which 21-deoxycortisol is the prime candidate. Excess aldosterone results in hypertension. Androgen production may result in severe virilisation in the female: hirsutism, voice deepening, hair recession and clitoromegaly. Oestrogen production may result in gynaecomastia in the male and in oestrogenisation of postmenopausal females. When there is no oversecretion of any hormonal steroids, the patient may present with loin pain, or a mass may be detected unexpectedly during a tomographic scan for unrelated reasons. Such tumours thus tend to be large and the time course of their growth and the evolution of their steroid production remains unknown.

Post surgery for adrenocortical carcinoma, steroids identified as increased may be used to monitor completeness of tumour removal and to detect tumour recurrence. The same pattern is often preserved on recurrence, but there are clear differences in some cases, probably due to differences in the selection of the cell clones that proliferate in the secondaries. Some secondaries with histological evidence that they are adrenocortical tumour tissue show no evidence of steroid secretion. Use of mitotane results in profound suppression of 5α-reductase 2 and 20β -hydroxysteroid dehydrogenase and induction of CYP3A4 (6β-hydroxylation). These effects can be taken into account when checking for the reappearance of steroids

that were increased before surgery. Since mitotane is invariably used with hydrocortisone, any steroid increase is likely to be autonomous. Androgen metabolites tend to be very low during mitotane treatment, but women of reproductive age still show evidence of cycling.

Glucocorticoid treatment

Oral treatment with cortisol or synthetic analogues results in suppression of endogenous cortisol production. Use of inhaled corticosteroids above a threshold dose will cause adrenal suppression. Glucocorticoid-containing skin creams can cause virtually complete adrenal suppression, especially if used extensively on broken skin or if the agent is especially potent (eg: clobetasol propionate). Use of long-acting depot preparations, such as of triamcinolone, can result in unexpectedly prolonged and profound suppression. If glucocorticoid treatment exceeds normal physiological levels, symptoms of Cushing's syndrome are produced. This may result in total abolition of ACTH-dependent adrenal steroid production. When investigating causes of adrenocortical insufficiency in patients who have been on hydrocortisone (cortisol) treatment, one protocol is to substitute dexamethasone and give depot synacthen (synthetic ACTH). Steroid secretion usually takes several days to show increase. Short withdrawal of treatment prior to investigation is thus uninformative, both because the adrenal will not have recovered from suppression and because this secondary effect cannot be differentiated from a primary adrenal defect.

Liver disease

Liver diseases which result in restricted venous flow, leading to portal hypertension, show consistent changes in the urinary steroid profile. These comprise increase of DHA metabolites, especially androstenetriol and of the cortisol metabolite α-cortolone. We have found this to be common to cirrhosis due to alcoholism or hepatitis C, congenital hepatic fibrosis, haemochromatosis and Wilson's disease.

Epilepsy

Children with epilepsy seem to have a high frequency of presentation with signs of precocious adrenarche. Urine findings generally support precocious adrenarche, with increased DHA and DHA metabolites but with a disproportionate rise in the metabolites. This suggests that 16α-hydroxylase in adrenals and/or liver is induced by their drug treatment.

Changes in body mass

Severe weight loss, as in anorexia nervosa, is associated with relative

decrease of 5α- reduced compared with 5β-reduced urine steroid metabolites. To some extent, the opposite is true for weight increase, although the effects of morbid obesity are surprisingly small. There is a weak correlation between urinary steroid output and body mass. Changes of insulin sensitivity likewise result in no clear changes in the urinary steroid profile. Extreme insulin resistance and insulin sensitization by use of metformin are two situations in which major change is not found.

Attribution. This text combines information published by the author and others, together with the unpublished experience and opinions of the author. It is provided for information only: no legal liability is accepted.

Norman Taylor

Director, SAS Steroid Profiling Service

Department of Clinical Biochemistry
King's College Hospital
London SE5 9RS

0203 299 4131 (direct line)
norman.taylor1@nhs.net

Feb 2014