Papers and Originals

Androgen Metabolism in Man—Some Current Concepts*

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Although the reaction proposed by Zimmermann (1935) enabled much important work to be done in assessing androgenic function in man, more specific techniques are required to gain precise information. Callow *et al.* (1938) applied Zimmermann's reaction to the assay of urinary 17-oxosteroids, then known as 17-ketosteroids, and they showed a fair correlation with the available data on the androgenic potency of urine extracts. However, any assay designed as a guide to individual androgenic status can hardly be regarded as adequate if some eunuchoidal men and virilized women have apparently normal results. Technical developments have permitted considerable advances in the field of androgen metabolism: these include the use of isotopically labelled tracer steroids and gas–liquid chromatography with its extremely sensitive means of measurement.

To understand the significance of these recent advances it is first necessary to have an idea of the androgenic potency of the compounds which may be encountered (Table I). Unfortunately many important data are lacking, particularly concerning the situation in man. With few exceptions, androgenic potency has been determined in chickens or small laboratory animals, with discordant results; but useful information has accrued from study of the effects of steroids in cancer patients (Segaloff, 1957). In addition, data have been abstracted from Dorfman and Shipley (1956), Short (1960), and Segaloff and Gabbard (1962). Testosterone and androstenedione are generally considered to be the most important androgenic steroids secreted. In man dehydroepiandrosterone appears not to be a potent androgen, and it would be reassuring to have better evidence that androstenedione is in fact markedly androgenic. Included in Table I are at least three steroid metabolites that may be androgenically important.

TABLE I.—*Androgenicity of C19-steroids*

Testosterone	+++	Etiocholanolone	0
Androstenedione	?++	Epiandrosterone	0
Dehydroepiandrosterone	?+	Androstane-3α,17β-diol	++
11β-hydroxyandrostenedione	0	Etiocholane-3α,17β-diol	0
Epitestosterone	0	Androst-5-ene-3β,17β-diol	++
Androsterone	+	Androst-5-ene-3β,17α-diol	±

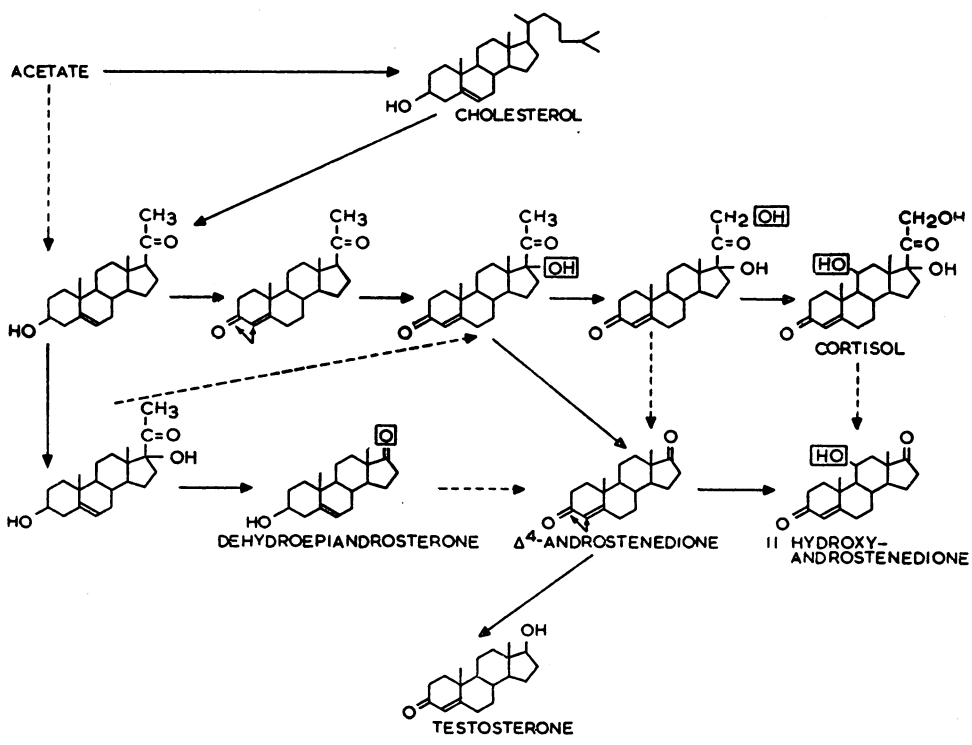

FIG. 1.—Scheme of C19-steroid biosynthesis.

Biosynthesis

The ways in which androgens are synthesized and converted to oestrogen in the various ductless glands must next be considered. Steroid biosynthesis is effected by a specific series of enzymes in the presence of co-factors, particularly nicotinamide-adenine dinucleotide and its phosphate. The overall synthesis of androgens and C19-steroids by the adrenal is indicated in Fig. 1. Dehydroepiandrosterone is synthesized from acetate via pregnenolone and 17α-hydroxypregnenolone. An important recent finding is that dehydroepiandrosterone sulphate is also directly synthesized from pregnenolone sulphate, and this may in fact be the predominant form of this steroid (Baulieu, 1965; Killinger and Solomon, 1965). It is probably pertinent that sulphates capable of hydrolysing dehydroepiandrosterone sulphate are widely distributed in the body (Warren and French, 1965). Androstenedione is formed by two pathways: from dehydroepiandrosterone, and, more importantly, via progesterone and 17α-hydroxyprogesterone (for references see Prunty, 1964a). In principle this synthesis, and the conversion of androstenedione to testosterone, is similar in the ovary and testis. However, in normal glands the

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capacity to convert androstenedione to 11β-hydroxyandrostenedione is thought at present probably to be restricted to the adrenal (see Prunty, 1964b) ; Dorfman *et al.*, 1965a).

The further conversion of androgen to oestrogen is of major importance in the human ovary (Fig. 2) and probably of minor importance in the adrenal and testis (see Prunty, 1964c ; Neher *et al.*, 1965). The role of the corpus luteum in the secretion of progesterone is clear, but the other loci of steroid biosynthesis in the human ovary are not as yet very well defined. The follicular tissue is important in the synthesis of androgen and oestrogen, but stromal tissue and the corpus luteum also appear to be the sources of some oestrogen (Rice *et al.*, 1964a, 1964b ; Short, 1964).

Pituitary trophic hormones play their part in stimulating steroid biosynthesis. Thus, in the case of the adrenal, the major effect of corticotrophin (A.C.T.H.) is to stimulate the production of progesterone from cholesterol (Fig. 1). The *in vitro* production of 11β-hydroxyandrostenedione is also stimulated by A.C.T.H., but this hormone is without effect on synthesis of other C19-steroids (Cohn and Mulrow, 1963). There is *in vivo* evidence that A.C.T.H. requires augmentation with another pituitary factor to increase overall C19-steroid production (Mills *et al.*, 1962). In the ovary luteinizing gonadotrophin increases synthesis of progesterone, testosterone, and oestrogen in varying ovarian tissues, and it increases the aromatization of the A ring of androstenedione. At present the role of follicle-stimulating hormone is more problematical (Kaiser, 1964 ; Rice *et al.*, 1964a, 1964b ; Dorfman *et al.*, 1965a). In the testis, too, testosterone synthesis is augmented by luteinizing hormone.

Metabolism

To interpret the *in vivo* evidence concerning abnormalities of androgen metabolism, one must be acquainted also with the way in which the C19-steroids produced in the body are metabolized. Reactions frequently involved are the reduction of the Δ_4-3-oxo grouping of ring A ; additional hydroxylation at, for example, carbons 6, 11, and 16 ; and in almost every case conjugation as glucuronide or sulphate. A 17-hydroxyl group may be oxidized to a 17-oxo group. In Table II are indicated many of the metabolic products of C19-steroids: it

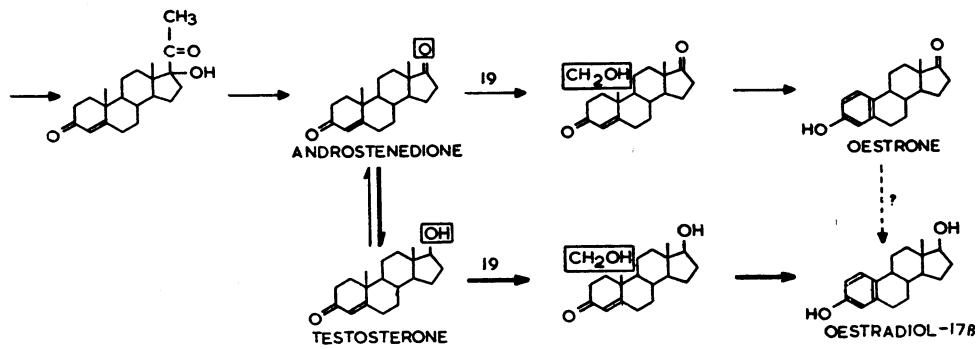

FIG. 2.—Ovarian synthesis of oestrogen from androgen.

TABLE II.—*Urinary Metabolites of C19-steroids*

Precursor	Metabolite	% Conversion	Glucoronide or Sulphate	17-Oxosteroid	References
Testosterone	Androsterone	25–50	GS	+	2, 6, 18
	Etiocholanolone		GS	+	
	Etiocholane-3α,17β-diol	~2	G		2
	Androstane-3α,17β-diol	~1	G		2
	Epiandrosterone	~1	S	+	2
	Androst-16-en-3α-ol	~0·4	G		6
	3α,18,dihydroxyandrostan-17-one	~0·3	G	+	11
	Testosterone	0·2–1	G		2, 7, 13, 14
	Testosterone	Traces	—		7
	11β-Hydroxytestosterone	„	G		19
	6α and 6β-Hydroxytestosterone	„	G		
Epitestosterone	Epitestosterone		G		4, 16
Androstenedione	Androsterone	25–30	GS	+	2, 9, 10
	Etiocholanolone		GS	+	
	Epiandrosterone	~1	S	+	2
	Etiocholane-3α,17β-diol	~0·8	G		2
	Androstane-3α,17β-diol	~0·3	G		
	Testosterone	~0·1	G		2, 7, 14
	Epitestosterone	~0·1	G		4
Dehydroepiandrosterone	Androsterone	17–40	GS	+	1, 6, 21
	Etiocholanolone		GS	+	
	Dehydroepiandrosterone	5–10	S	+	1, 6, 21
	„	Traces	G	+	20
	16α-Hydroxydehydroepiandrosterone		S	+	17
	Androst-5-ene-3β,17β-diol	~1	S		1
	Androst-5-ene-3β,16α,17β-triol	~0·4	S		1
	Androst-16-en-3α-ol	~0·4	G		5, 6, 8, 12
	Testosterone	~0·1	G		15
Dehydroepiandrosterone sulphate	Dehydroepiandrosterone	~10	S	+	1
	Androsterone	~10	GS	+	1
	Etiocholanolone		GS	+	
	Androst-5-ene-3β,17β-diol	~2	S		1
	Androst-5-ene-3β,17β-triol	~1	S		1
11β-Hydroxyandrostenedione	11β-Hydroxyandrosterone	~50	GS	+	3
	11β-Hydroxyetiocholanolone	~5	GS	+	
	11-oxo-Etiocholanolone	~12	G	+	
	11-oxo-Androsterone	~3	G	+	
Androst-16-en-3α-ol	Androst-16-en-3α-ol	6	G		6

References to Table II: 1, Baulieu (1965); 2, Baulieu and Mauvais-Jarvis (1964); 3, Bradlow and Gallagher (1957); 4, Brooks and Guiliani (1964); 5, Brooksbank (1962); 6, Bulbrook *et al.* (1963); 7, Camacho and Migeon (1964); 8, Cleveland and Savard (1964); 9, Dorfman (1959); 10, Dorfman *et al.* (1950); 11, Fukushima and Bradlow (1962); 12, Gower (1963); 13, Horton *et al.* (1965); 14, Korenman and Lipsett (1964); 15, Korenman and Lipsett (1965); 16, Korenman *et al.* (1964); 17, Prunty (1964d); 18, Rubin *et al.* (1954); 19, Schubert *et al.* (1964); 20, Siiteri *et al.* (1963); 21, Vande Wiele and Lieberman (1960).

also shows the approximate percentage conversion of a precursor to a particular metabolite, and the usual form of conjugation. Twenty-one different steroid metabolites are represented, but their importance in terms of androgenicity does not necessarily correlate with their quantity. A few are known to be androgenic (see Table I). A number of these metabolites—particularly androsterone, etiocholanolone, dehydroepiandrosterone, and the 11-oxy C19-steroids—contribute to the quantity of 17-oxosteriods measured in the urine. One must also recall that a small proportion of urinary 17-oxosteroid excretion is composed of 11-oxygenated C19-steroid metabolites of cortisol and other C21-steroids (Prunty, 1964e). Now that we have a good knowledge of the steroids contributing to the 17-oxosteroids, customary techniques for their total evaluation have proved inadequate even for many clinical purposes, largely owing to the presence of non-specific interfering substances in relatively crude extracts (Goldzieher and Axelrod, 1962; Ernest *et al.*, 1964). Precision may be greatly improved by quantitative individual estimation of many of the steroid metabolites.

Table II shows considerable variation in the form of conjugation of the metabolites. Androsterone and etiocholanolone are excreted predominantly as glucuronides, and only about one-tenth is present as sulphates (Kellie and Smith, 1957; Brooks, 1958). On the other hand, dehydroepiandrosterone is excreted predominantly as sulphate, and testosterone as glucuronide.

Normal Quantitative Aspects

A synopsis of the current knowledge of the plasma levels and urinary excretion of the C19-steroids in normal circumstances is given in Table III. In many cases, owing to variation between observers and paucity of data, only approximate figures can be given. In the male the predominant androgen in the plasma is clearly testosterone; and in the female androstenedione, and even androsterone, may well be important. At present the reason for the large secretion and high plasma level of dehydroepiandrosterone sulphate is unknown.

Though quite different techniques have been employed by various observers, there is widespread agreement on the plasma-testosterone level in the male, the overall mean being approximately 0.81 μg./100 ml. Agreement is less good for the female, but the values given from our laboratory (mean 0.05 μg./100 ml.) agree closely with those of Lobotsky *et al.* (1964), Horton and Tait (1965), and Van den Molen *et al.* (1965). The correlation of plasma-testosterone levels with 17-oxosteroid excretion is poor (Lobotsky *et al.*, 1964). In males the plasma-testosterone rises sharply at puberty and declines by the age of 80 years; in females there seems to be no consistent variation during the menstrual cycle.

There is also general agreement on the amount of testosterone glucuronide excreted in the urine, the overall averages being 100 μg./day in males and 9 μg./day in females. Although the excretion in the male rises rapidly at puberty, it declines at the age of 45, which is earlier than the decline of the plasma-testosterone level (Vermuelen, 1966). (Note that the measurement of either plasma-testosterone or the excretion of the glucuronide in normals provides a good distinction between the sexes.) Also excreted in the urine is a comparable amount of the glucuronide of the inert epitestosterone, the biological importance of which remains to be assessed.

A considerable difference is found between the renal excretion of the steroid glucuronides and that of sulphates. The renal clearance of the glucuronides approximates to the glomerular filtration rate, while the clearance of the sulphates is only about one-tenth of this (Kellie and Smith, 1957).

Secretion and Production of Steroids

In practice, determination of the secretion rate of the adrenocortical hormone cortisol has proved particularly useful (Brooks *et al.*, 1963). The problem involved in this particular case is comparatively simple. Naturally attempts are being made to apply similar principles to the determination of the rate of secretion of the androgens.

In the most simple circumstances an administered isotopically labelled steroid may be assumed to mix with, and behave in the same way as, the endogenously secreted hormone which is distributed in the body in a single anatomical compartment. The secretion rate of the hormone, assuming that its entry into and exit from the compartment are in equilibrium, may be determined by the product of the rate of irreversible clearance from the plasma (metabolic clearance rate) of the administered tracer and the plasma concentration of the endogenous hormone. Alternatively, if a metabolite originating solely from the endogenous hormone can be isolated from the urine, the rate of secretion can be found from knowledge of the amount of administered radioactivity and the cumulative specific activity of the metabolite, provided the urine is collected for sufficient time to permit recovery of all, or nearly all, the isotope injected.

Attempts to measure the secretion rates of C19-steroids have revealed that the conditions are very much more complex than this simple ideal. In the first place several anatomical compartments appear to be involved, so that access to blood alone is not representative of the changes taking place. Secondly, there is considerable interconversion of the steroids concerned. Tait and Horton (1964) and Horton and Tait (1966) have made much progress in the mathematical solution of the problem of multiple compartments in relation to the secretion of testosterone.

The interconversion of C19-steroids is depicted in Fig. 3. There is no urinary metabolite specific for a single C19 precursor—for instance, in the case of dehydroepiandrosterone the administration of ^{3}H-dehydroepiandrosterone is often found to result in a lower specific activity in the urine of dehydro-

TABLE III.—*Plasma Levels and Urinary Excretion of C19-steroids*

	Plasma (μg./100 ml.)		References	Urine (μg./day)		References
	M	F		M	F	
Testosterone	0·81* (0·67–1·1)	0·05* (0·02–0·09)	2, 3, 6, 8, 9, 14, 15, 17, 23, 24	~1·1	~0·7	24
Androstenedione	~0·04	~0·1	8, 9	—	—	
Dehydroepiandrosterone	1·3	1·0	4, 9, 14	—	—	
Testosterone glucuronide	~0·3	~0·1	2, 24	72* (33–120)	12* (7–18)	7, 11, 16, 22, 24, 25
Epitestosterone Gl.	—	—		182*	36*	
Dehydroepiandrosterone-SO_4	~150	~100	1, 5, 10, 15, 19, 20, 26	2,900	1,100	12, 18, 21
Androsterone	~0·3		4, 14	—	—	
Androsterone-SO_4	~40		1, 5, 10, 20	3,600	2,200	12, 18, 21
Androsterone-glucuromide	~2		13			
Etiocholanolone	~0·5		4, 14	—	—	
Etiocholanolone-SO_4	~9		1	4,000	2,600	12, 18, 21
Etiocholanolone-glucuromide	~2		13			

* Values in our laboratory (see Brooks, 1964; Lim and Brooks, 1965).

References to Table III: 1, Baulieu (1960); 2, Burger *et al.* (1964); 3, Casey (1965); 4, Cohn *et al.* (1961); 5, Conrad *et al.* (1961); 6, Dorfman *et al.* (1963); 7, Futterweit *et al.* (1964); 8, Horton and Tait (1965); 9, Hudson *et al.* (1965); 10, Hudson and Oertel (1961); 11, Ibayashi *et al.* (1964); 12, James *et al.* (1962); 13, Kellie and Smith (1957); 14, Kirschner *et al.* (1965); 15, Lamb *et al.* (1964); 16, Lim and Dingman (1965); 17, Lobotsky *et al.* (1964); 18, Mahesh *et al.* (1964); 19, Migeon (1955); 20, Migeon and Plager (1955); 21, Prunty (1964f); 22, Rosner *et al.* (1965); 23, Surace *et al.* (1966); 24, Van den Molen *et al.* (1965); 25, Vermuelen (1966); 26, Wieland *et al.* (1965).

epiandrosterone than of androsterone and etiocholanolone (Fig. 4) (Brooks and Prunty, 1962 ; Vande Wiele *et al.*, 1963). This can now largely be explained on the basis of a considerable secretion of dehydroepiandrosterone sulphate, which results in dilution of the conjugate derived from the metabolism of free dehydroepiandrosterone.

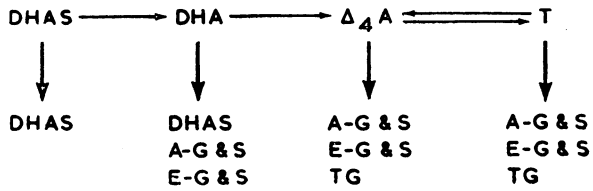

FIG. 3.—Interconversion *in vivo* of androgens. Note that dehydroepiandrosterone sulphate (DHAS) in the urine is derived from both dehydroepiandrosterone (DHA) and its sulphate ; andosterone (AG & S) and etiocholanolone (E–G & S) are common metabolites of DHA, androstenedione (Δ4A), and testosterone (T) ; and testosterone glucuronide (TG) has more than one source.

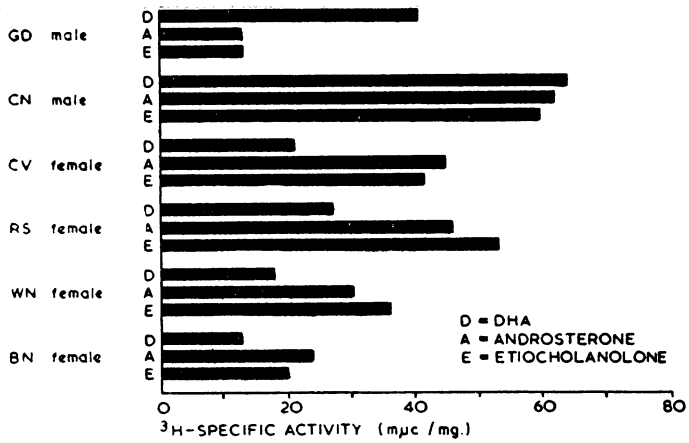

FIG. 4.—Specific activity of urinary metabolites after administering ^{3}H-dehydroepiandrosterone. Note the specific activity of dehydroepiandrosterone in the females is less than that of androsterone and etiocholanolone ; in the male patient CN it is probable that the three specific activities are similar owing to "dilution" of androsterone and etiocholanolone from testicular testosterone. Patient GD had Addison's disease (see text).

The first patient in Fig. 4 had Addison's disease treated with cortisone, thus having a low rate of endogenous adrenocortical activity, and he could therefore be assumed to secrete minimal amounts of dehydroepiandrosterone sulphate. He was given 15 mg. each of dehydroepiandrosterone and androstenedione daily by intramuscular injection, and, simultaneously, tracer amounts of ^{3}H-dehydroepiandrosterone and of ^{14}C-androstenedione. The specific activity, with respect to ^{3}H, of the dehydroepiandrosterone in the urine was much greater than that of the androsterone and etiocholanolone, indicating that some of these latter metabolites originated from another source, in this case the androstenedione. Since in this patient the major source of the two precursors was external, it was possible to obtain a measure of their "secretion rate." For dehydroepiandrosterone this was obtained from the ^{3}H in the urinary dehydroepiandrosterone ; the "secretion rate" of the androstenedione could then be calculated from the ^{14}C specific activity of the androsterone and etiocholanolone after deducting the amount of these metabolites originating from dehydroepiandrosterone. The "secretion rate" of dehydroepiandrosterone calculated was 18.4 mg./day, and for androstenedione 16.0 mg./day. These values are in reasonable agreement with the amounts administered, especially taking into account the small quantities of endogenously secreted steroid which were presumably present.

Further inspection of Fig. 3 shows that interconversion of C19-precursors takes place. In the case of testosterone the urinary glucuronide arises from more than one source. Apart from the metabolism of testosterone to the glucuronide, the glucuronide has also been found to be formed by the metabolism of dehydroepiandrosterone (Korenman and Lipsett, 1965) and also particularly of androstenedione (Camacho and Migeon, 1964 ; Korenman and Lipsett, 1964). The situation is further complicated, from the biological standpoint, in that when androstenedione is converted to testosterone glucuronide only a small portion appears at any time as free and active testosterone (Mahesh and Greenblatt, 1962), the major portion apparently being metabolized direct to the inactive glucuronide in the liver.

The above deductions concerning the fate of dehydroepiandrosterone and androstenedione were made from studies of the metabolism of tracer steroids. Complementary evidence of a biological nature also leads to the same conclusion.

The daily administration of 100 mg. of androstenedione to a male subject resulted in an increased excretion of testosterone glucuronide of 219 μg./day. If this had originated from endogenously secreted testosterone it would have represented a daily secretion of the precursor of at least 15 mg. The nitrogen balance of this patient, which was also being followed, showed no evidence of his exposure to this quantity of free testosterone (McSwiney *et al.*, 1964).

Since it may be possible, by the simultaneous administration of tracer precursors, to obtain estimates of their individual production in situations where the endogenous steroids are interconverted, such determinations have become known as "production rates," as opposed to the "secretion rates" of the particular steroids from the appropriate gland or glands (Vande Wiele *et al.*, 1963). For reasons already given, such a production rate may not truly indicate the amount of the free and active steroid to which the body has been exposed. Horton and Tait (1966) have concluded that in normal subjects dehydroepiandrosterone contributes little to the production of androstenedione and testosterone as measured by "blood-production rates." In the female the androstenedione appears to result from the secretion of androstenedione, and in the male the testosterone from the secretion of testosterone. They calculated, however, that about half the testosterone in the female, and half the androstenedione in the male, derives from conversion of androstenedione and testosterone respectively. An interpretation of available estimates of normal secretion and production rates of C19-steroids is summarized in Table IV. In the male testosterone is largely secreted primarily from the testis, but in the female it largely originates by conversion. Interconversions of dehydroepiandrosterone and of its sulphate result in greater production than secretion rates.

TABLE IV.—*Secretion and Production of C19-steroids (mg./day)*

	Secretion		Production		References
	M	F	M	F	
Testosterone	7	~0·14	~7	0·3–2·9	2, 4, 5, 6
Dehydroepiandrosterone-SO4	6	6	16	7–10	1, 3, 8
Dehydroepiandrosterone	3	2	—	5	3, 8
Androstenedione	~0·5	~3·5	—	3·3	4, 8
11β-Hydroxyandrostenedione	~2		—	—	7

References to Table IV: 1, Baulieu (1965) ; 2, Baulieu and Mauvais-Jarvis (1964) ; 3, Gurpide *et al.* (1965) ; 4, Horton and Tait (1966) ; 5, Hudson *et al.* (1965) ; 6, Kent and Acone (1966) ; 7, Prunty (1964g) ; 8, Vande Wiele *et al.* (1963).

Opinion is fairly unanimous that the production rate of testosterone in normal males by urinary estimates ("urinary production rate") is close to 7 mg./day (see Table IV ; and Korenman *et al.*, 1963 ; Horton *et al.*, 1965 ; Lim and Dingman, 1965) and in normal females close to 1.5 mg./day (*loc. cit.* ; and Prunty *et al.*, 1964 ; Korenman *et al.*, 1965). In comparative studies Hudson *et al.* (1965) have concluded that the blood-production rates in the male are about 6 mg./day, and in the female 0.78 mg./day, being in fact somewhat lower than the urinary values. In the male the production rate correlates well with plasma-testosterone levels (Davis *et al.*, 1965). It should be noted that, even in the male, the secretion of testosterone can contribute as little as about 3 mg./day to the 17-oxosteroids excreted.

Establishing the Origin of Androgens

In some normal and all abnormal circumstances one of the first requirements is to establish which glands are concerned in the *in vivo* synthesis of androgenic steroids. There are various methods of approach to this problem. A most direct but difficult one is to obtain blood from the venous drainage of the gland in question. In the case of the adrenal this can be done by catheterization, but the procedure itself is very likely to increase endogenous adrenocortical activity; in the case of the ovary admixture of uterine blood cannot be avoided. A second method is to study the effect of removal of the gland in question, or of a disease known to be confined to it. Less direct methods include suppression of adrenocortical activity by the administration of a corticosteroid, usually dexamethasone, or stimulation of it by A.C.T.H. In the case of the gonads, stimulation with chorionic gonadotrophin may be attempted; the testis may be suppressed by a potent androgen or oestrogen. Techniques of ovarian suppression require further development.

The Adrenal Cortex

Testosterone, dehydroepiandrosterone and its sulphate, androstenedione, and 11β-hydroxyandrostenedione have been found in higher concentration in adrenal venous blood than in peripheral blood, but this difference seems least marked in the case of testosterone (see Prunty, 1964h; Burger *et al.*, 1964; Baulieu, 1965; Casey, 1965; Wieland *et al.*, 1965). Adrenalectomy has been performed in cancer patients following oophorectomy, and then the plasma level of testosterone is greatly reduced (Riondel *et al.*, 1963; Horton and Tait, 1965). Administration of A.C.T.H. to normal subjects has resulted in increases of urinary testosterone glucuronide and of plasma-testosterone level and production rate, but these increases are not consistent; similarly, depression with dexamethasone and prednisone is not entirely consistent (see Fig. 6) (Korenman *et al.*, 1963, 1965; Lamb *et al.*, 1964; Hudson *et al.*, 1965; Kirschner *et al.*, 1965; Vermuelen, 1965). Nevertheless, there is evidence *in vivo* that some testosterone is of adrenal origin.

Normal values for the excretion of testosterone glucuronide have been obtained in Addison's disease (Ibayashi *et al.*, 1964; Vermuelen, 1966), and consistently raised values of excretion and plasma-testosterone level and production have been found in congenital adrenal hyperplasia (Burger *et al.*, 1964; Futterweit *et al.*, 1964; Casey, 1965; Korenman *et al.*, 1965; Lim and Dingman, 1965; Rosner *et al.*, 1965; Degenhart *et al.*, 1966) but the correlation with 17-oxosteroid excretion is not close; there is in this condition good suppression of testosterone with corticosteroid. Few observations are available in Cushing's syndrome with adrenal hyperplasia, but urinary testosterone glucuronide is increased (Ibayashi *et al.*, 1964; Vermuelen, 1966) and a high level of testosterone has been observed in the adrenal vein (Hudson *et al.*, 1963) and a low level in the peripheral plasma of such a patient after adrenalectomy (Lamb *et al.*, 1964). All three values of testosterone metabolism show increases in patients with adrenal carcinomas and adenomas of various types, but again the correlation with 17-oxosteroid excretion is not close (Futterweit *et al.*, 1964; Casey, 1965; Korenman *et al.*, 1965; Rosner *et al.*, 1965).

Hypopituitarism

As would inevitably be expected, patients with anterior hypopituitarism have low excretion of testosterone glucuronide (Ibayashi *et al.*, 1964; Lim and Dingman, 1965; Rosner *et al.*, 1965; Vermuelen, 1966) and low testosterone-production rates (Hudson *et al.*, 1965; Lim and Dingman, 1965). Males so tested have responded well to chorionic gonadotrophin.

The Testis

Quite clearly the testis is a potent source of testosterone. If interstitial-cell function is good the testosterone concentration is very much higher in testicular vein blood than in the peripheral blood (Fig. 5) (Hollander and Hollander, 1958; Hudson *et al.*, 1965). When an intravenous infusion of chorionic

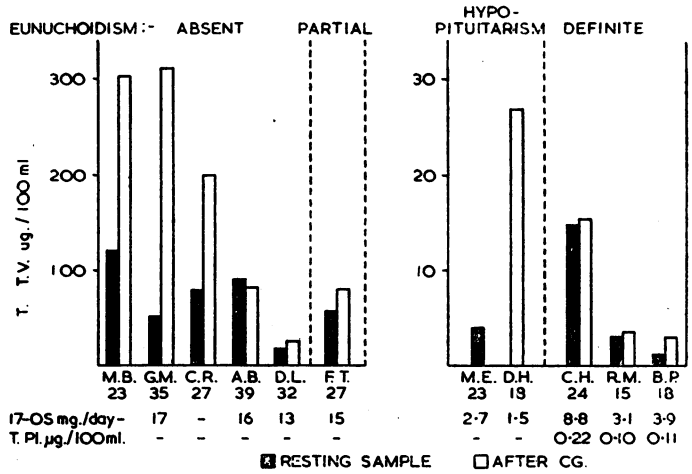

FIG. 5.—Concentration of testosterone in the plasma from the testicular vein before and after the administration of chorionic gonadotrophin to patients with testicular disease. Chorionic gonadotrophin was given as 2,000 units intramuscularly and one hour later 5,000 units intravenously during one-hour period. Note that right-hand scale is one-tenth of left-hand scale. T.T.V. indicates testosterone in testicular vein; T.Pl.= testosterone in peripheral plasma; and 17-OS=urinary 17-oxosteroids.

gonadotrophin was given to patients with testicular abnormalities there was a large increase in the testosterone concentration in some (Dupré *et al.*, 1964). It is difficult to measure precisely the testicular secretion rate of testosterone by direct means owing to the practical difficulty of eliciting the true blood-flow. Values between 2.1 and 3.4 mg./day were obtained, but these are likely to be less than the actual rates. Hudson *et al.* (1965) have had similar experience. Others have stimulated increases of urinary testosterone glucuronide, plasma testosterone, and testosterone production in normal males with chorionic gonadotrophin; but, where sought, responses in males with diseased testicles have usually been impaired (Fig. 5) (Horton *et al.*, 1965; Hudson *et al.*, 1965; Kirschner *et al.*, 1965; Korenman *et al.*, 1965; Lim and Dingman, 1965; Vermuelen, 1966).

Naturally, if interstitial-cell function is impaired, the level of testosterone may be extremely low in the spermatic vein blood (Fig. 5) (Hudson *et al.*, 1963) and low also in the peripheral blood; the excretion of testosterone glucuronide and testosterone production also fall markedly (Ibayashi *et al.*, 1964; Casey, 1965; Hudson *et al.*, 1965; Lim and Dingman, 1965; Rosner *et al.*, 1965). In castrates, too, testosterone glucuronide excretion is very low (Futterweit *et al.*, 1964; Dorfman *et al.*, 1965b; Tamm *et al.*, 1966). The plasma testosterone level can be increased by the administration of A.C.T.H. and depressed by dexamethasone in eunuchoidism (Fig. 6), suggesting a continuing adrenocortical contribution to the production of testosterone. The interrelation between testicular androgen secretion and the production of adrenocortical C19-steroids appears to be complex. Many patients with primary interstitial-cell failure have a tendency to excessive production of adrenal C19-steroids, both at rest and after stimulation with A.C.T.H. (Mills *et al.*, 1962; Clayton *et al.*, 1966). Steeno *et al.* (1966) also draw attention to this complex relationship.

The testicular component in the production of testosterone can be very largely suppressed by the administration of ethinyl-oestradiol (Dorfman *et al.*, 1965b); potent synthetic androgens, such as fluoxymesterone (Kirschner *et al.*, 1965) and 2α-methyldehydrotestosterone, also have this property (Davis

et al., 1965). It has been suggested that, at least in the case of the latter compound, the suppression of testosterone secretion is due to inhibition of luteinizing hormone secretion, because the depressing effect of the androgen can be overcome by the administration of chorionic gonadotrophin.

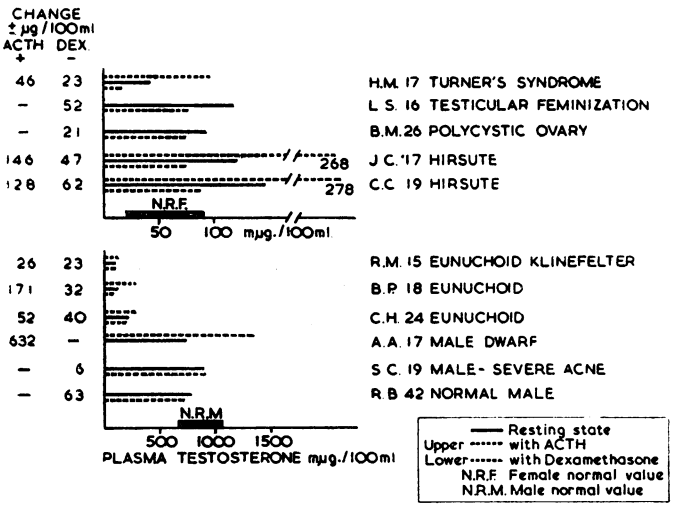

FIG. 6.—Plasma testosterone and its response to A.C.T.H. and dexamethasone (DEX) in various conditions. A.C.T.H. 20 i.u. intramscularly was given twice daily for four days, dexamethasone 2 mg. daily. Note that the upper scale is one-tenth the lower scale.

The Ovary

As its biosynthetic capabilities suggest, the ovary is an important potential source of androgen. However, attempts to increase testosterone from this source by giving chorionic gonadotrophin to normal women have not been too successful (Korenman *et al.*, 1963, 1965 ; Lamb *et al.*, 1964 ; Hudson *et al.*, 1965 ; Vermuelen, 1966), though the duration and regulation of dosage may have something to do with this. Bilateral oophorectomy reduces the plasma testosterone to low normal values (Hudson *et al.*, 1963 ; Riondel *et al.*, 1963 ; Lamb *et al.*, 1964 ; Lobotsky *et al.*, 1964 ; Horton and Tait, 1965). As may well be expected, a large variety of ovarian tumours which produce virilism have been shown *in vivo* to be capable of synthesizing androstenedione and testosterone (see Segre *et al.*, 1964), including even a granulosa-cell tumour (Mills *et al.*, 1959).

A very high plasma testosterone has been noted *in vivo* in a case of hilar-cell tumour (Dorfman *et al.*, 1963 ; and testosterone glucuronide excretion was increased in patients with arrhenoblastoma and lipoid ovarian tumour (Rosner *et al.*, 1965), although urinary 17-oxosteroids may often not be raised. The concentration of testosterone in ovarian-vein blood from two virilizing tumours was also very high (Hudson *et al.*, 1963 ; Simmer, 1964).

Polycystic Ovary Syndrome

A particular challenge in the field of androgen metabolism has been the syndrome of polycystic ovaries, which is often associated with hirsutism and even severe virilism. Investigation of the steroid content of the ovary, particularly the follicular fluid (Mahesh, 1965 ; Short, 1965), and *in vitro* steroid biosynthesis have shown increases in ovarian C19-steroids, including dehydroepiandrosterone, androstenedione, and testosterone. Enzymatic abnormalities in the aromatization of ring A of the androgen (Fig. 2) and deficiency of 3β-dehydrogenase activity in the conversion of pregnenolone (Fig. 1) have been postulated as possible lesions. Apparent simultaneous deficiency of pregnenolone in ovary and adrenal cortex has been observed (Axelrod *et al.*, 1965). The general opinion has been that, although androgen synthesis in the ovary is excessive, testosterone as such is not secreted into the ovarian vein (Mahesh, 1965 ; Short, 1965), but there are recent indications that small quantities might be present (Simmer, 1964). On the other hand, there is also evidence for the continued secretion of testosterone into the adrenal vein (Casey, 1965).

Observations on the steroid metabolism have resulted in varied findings, suggesting a number of subvarieties of the syndrome. Urinary 17-oxosteroids vary from low normal to slightly raised amounts: in some patients their excretion is easily suppressed by corticosteroids ; in others there is little suppression (Fig. 7) ; and some are particularly responsive to A.C.T.H. (Prunty *et al.*, 1964). The excretion of individual 17-oxosteroids also varies—for example, there is a tendency for an increase of 11-deoxy-17-oxosteroids and 11β-hydroxyandrosterone. The latter is presumed to be associated with increased adrenocortical activity in androgen production. However, it is difficult to be sure how much the adrenal, in addition to the ovary, contributes to excess androgen in these patients (Brooks and Prunty, 1960 ; Mahesh, 1965).

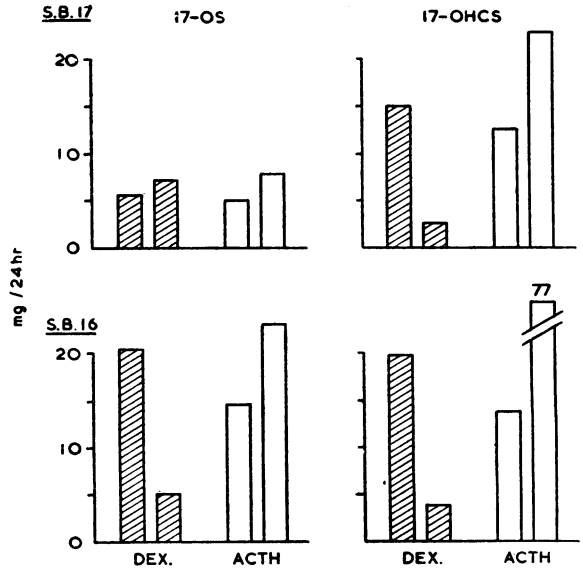

FIG. 7.—Response of two patients who were severely virilized with polycystic ovaries to dexamethasone (DEX, 6 mg. daily for three days) and to A.C.T.H. (20 i.u. intramuscularly twice daily for four days). 17-OS=urinary 17-oxosteroids ; 17-OHCS=urinary 17-hydroxycorticosteroids.

Studies of testosterone metabolism only partly clarify this difficult situation. The plasma level, production rate, and glucuronide excretion are increased in many but not all patients (Figs. 6 and 8) (Dorfman *et al.*, 1963 ; Dignam *et al.*, 1964 ; Futterweit *et al.*, 1964 ; Ibayashi *et al.*, 1964 ; Casey, 1965 ; Korenman *et al.*, 1965 ; Lim and Dingman, 1965 ; Surace *et al.*, 1966). In Fig 8 there is little correlation with the degree of hirsutism or virilism ; the large number of normal values with dexamethasone suppression is compatible with an adrenocortical origin of some abnormal•androgen.

The experiment in Fig. 9 shows that in the patient studied, and in similar patients (Brooks *et al.*, 1966), all the testosterone production can be due to conversion of androstenedione. In this example testosterone production is indeed normal, but that of androstenedione almost certainly increased. The testosterone originates from androstenedione because the specific activity of the testosterone glucuronide with respect to ^{3}H, arising from the tracer ^{3}H-androstenedione administered, is not less than that of the androsterone and etiocholanolone ; since in this case the excretion of dehydroepiandrosterone sulphate was very small, the adrenal component having been suppressed with

dexamethasone, these latter metabolites are believed to have originated very largely from androstenedione.

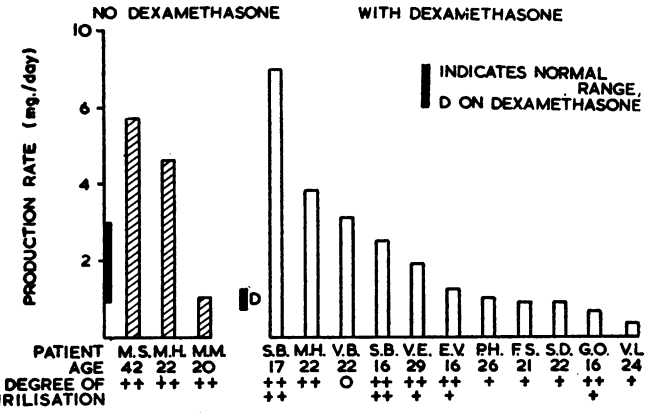

FIG. 8.—Production rate of testosterone in patients with polycystic ovaries. Dexamethasone 4 mg. was given daily (6 mg. daily in S.B.16 and S.B.17). The unsuppressed normal values taken from data of others (see references in text), and during suppression from Korenman *et al.* (1963) and Prunty *et al.* (1964). These comparisons are tentative.

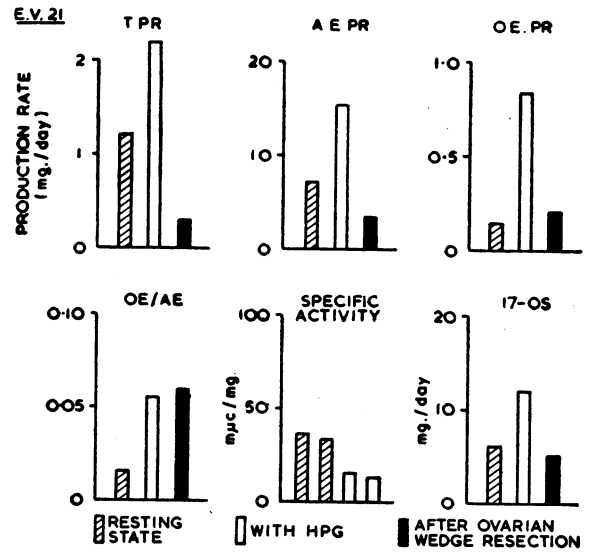

FIG. 9.—Simultaneous production rates of testosterone, androstenedione, and oestrogen determined with ^{14}C-testosterone, ^{3}H-androstenedione, and ^{14}C-oestrone. Oestrogen production was estimated from specific activity of oestriol. Dexamethasone 4 mg. daily was given throughout, and human pituitary gonadotrophin 2,000 I.R.P. units on the second day before and on each of the two days during which production rate was determined. PR=production rate; T=testosterone; AE=androstenedione; OE=oestrogen; 17-OS=17-oxosteroid excretion; TG=testosterone glucuronide; A=androsterone.

As observed with 17-oxosteroids, corticosteroid administration sometimes—but not always—diminishes the plasma level and production of testosterone (Figs. 6 and 8) (Dorfman *et al.*, 1963; Dignam *et al.*, 1964; Hudson *et al.*, 1965; Korenman *et al.*, 1965).

The effects of gonadotrophin are especially important in the polycystic ovary syndrome. It has sometimes been possible to increase plasma testosterone concentration with chorionic gonadotrophin (with luteinizing hormone activity) (Dorfman *et al.*, 1963; Dignam *et al.*, 1964; Hudson *et al.*, 1965). On the other hand, human menopausal gonadotrophin, which largely consists of follicle-stimulating hormone, has been found *in vitro* to stimulate androstenedione biosynthesis in the polycystic ovary (O'Donnell and MacArthur, 1965); *in vivo* increased excretion of 11-deoxy-17-oxosteroids has been induced with human pituitary gonadotrophin, while the adrenal was suppressed with dexamethasone (Mahesh, 1965).

The effects observed with a human pituitary gonadotrophin are seen in Fig. 9 (Brooks *et al.*, 1966). Testosterone, androstenedione, and oestrogen productions were all increased, although the ratio oestrogen/androstenedione became more normal. Crooke *et al.* (1963) observed that human pituitary gonadotrophin increased oestrogen excretion, and that after the additional administration of chorionic gonadotrophin the steroid pattern within the ovary became more normal, so that ovulation became possible (Edwards, 1964; Short, 1965). It is uncertain how important the chorionic gonadotrophin is in achieving this reversal of steroid production towards normal. While primarily consisting of follicle-stimulating hormone, the human pituitary gonadotrophin preparations are known to contain small proportions of luteinizing hormone (Crooke, 1964), and this may be important in the above experiment. Widespread experience has shown that steroid metabolism may return to normal in some patients, and ovulation occur in many, after the operation of ovarian wedge resection, but the reason has remained a mystery. This response is seen dramatically in Figs. 9 and 10. The reversion towards normal that takes place with gonadotrophin administration renders the theory of congenital enzymatic blocks unlikely, and suggests an error in the gonadotrophin mechanism (Short, 1965) despite the fact that the urinary excretion of total gonadotrophin in these patients is within normal limits (Prunty *et al.*, 1964). It would seem that ovarian wedge resection somehow modifies this mechanism.

The possibilities of suppressing the abnormal ovarian steroid production in the polycystic ovary syndrome deserves further study. Oestrogens have suppressing effects on the urinary excretion of 17-oxosteroids (Mahesh, 1965; Prunty, 1964j). An interesting compound in this connexion is 1α-allylthiocarbamoyl-2-methylthiocarbamoyl-hydrazine (I.C.I. 33828); administration of this substance to two severely virilized patients depressed testosterone production to a greater extent than did dexamethasone (Fig. 10). The 17-oxosteroid excretion and the "apparent" production of dehydroepiandrosterone also fell (Prunty *et al.*, 1964), and these changes correlated with a considerable inhibition of total urinary gonadotrophin excretion. This fact, together with the observation that the compound has a depressing effect on ovarian oestrogens originating from normal ovaries (Bell *et al.*, 1962), suggests that its action is indeed upon the ovary rather than on the adrenal, although its exact effect is obscure (Brown, 1963).

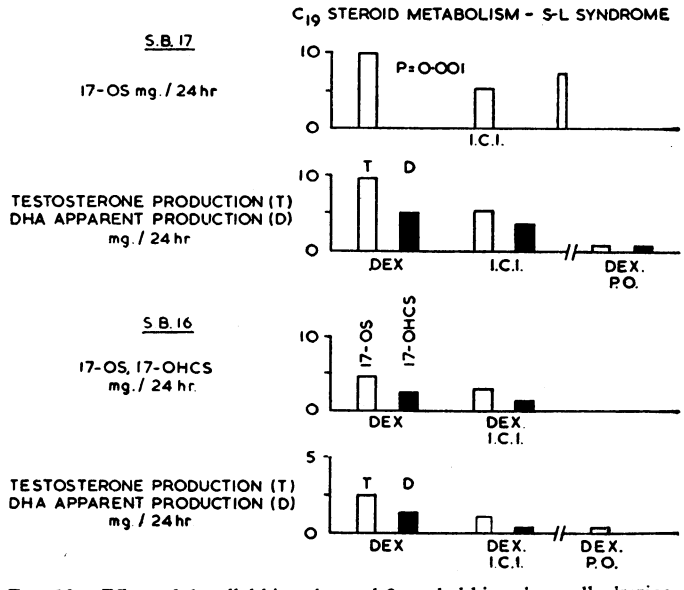

FIG. 10.—Effect of 1α-allylthiocarbamoyl-2-methylthiocarbamoylhydrazine (I.C.I. 33828) on androgen production in two patients with the polycystic-ovary syndrome. The patients had 6 mg. dexamethasone daily as shown (DEX); S.B.17 had 200 mg. I.C.I. 33828 daily and S.B.16 had 300 mg. PO=effect of bilateral subtotal ovariectomy; 17-OS=urinary 17-oxosteroids; 17-OHCS=urinary 17-hydroxycorticosteroids.

Conclusion

It must be concluded that the complexity of gonadal adrenal interaction still presents a challenge to our understanding of androgen metabolism, especially in the polycystic-ovary syndrome. Space has not permitted consideration of the more difficult problem of "simple hirsutism," in which some of the abnormalities in the polycystic-ovary syndrome are also found (Fig. 6) (Brooks and Prunty, 1960 ; Mahesh and Greenblatt, 1964).

Virilization and precosity in congenital adrenal hyperplasia have also been neglected, but recent reviews on this topic are available (Brooks, 1962 ; Cope, 1965 ; Prunty, 1964k ; Segre *et al.*, 1964).

The new ability to study testosterone metabolism in detail is leading to big advances in the understanding and diagnosis of abnormalities of the adrenals and gonads ; at least it is now possible to distinguish clearly between the sexes, and to recognize eunuchoid individuals by direct biochemical means.

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Adrenalectomy for Disseminated Breast Cancer

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The incidence of breast cancer in women in England and Wales has risen progressively for the past 40 years, and during 1964 9,860 women died of it. The cause of death in these cases is metastatic spread of the disease.

Huggins and Bergenstal (1952) showed that control of metastases from breast cancer can be achieved in a proportion of patients by adrenalectomy. This report is based on a personal series of 348 patients (including three males) with a disseminated breast cancer, submitted to bilateral adrenalectomy and gonadectomy during the past 14 years.

The operation is effective in about half of those submitted to it, and the results in hormone-dependent tumours are unsurpassed by any other form of treatment as regards degree of response and length of period of remission, and only equalled by the results of hypophysectomy.

Clinical Material

The ages of the patients varied from 24 to 76 years, but the majority were in the fourth and fifth decades. The series included 24 patients with bilateral mammary cancer and nine with pregnancy or lactational cancer. There is no evidence that the younger age group responded differently from the older; neither was success or failure in any way related to the pre- or post-menopausal state of the patient.

The operative mortality, defined as death within one month of the operation, was 4.3% in the overall series and 1% in the last 100 patients. The technique of the operation in all cases was by the posterior route through the bed of the 11th and 12th ribs. In almost all patients the operation was done in two stages: left adrenalectomy and bilateral oophorectomy followed one week later by right adrenalectomy. No correlation was observed between the histological variety of breast cancer and the response to adrenalectomy.

Site of Metastases.—The commonest site of metastases was the skeleton; in many patients both skeletal and visceral metastases were present, in others lymph nodes were involved, and in some there were cutaneous recurrences. The distribution of metastases is shown in Table I. The histological examination of the ovaries and adrenals showed that 40% of the adrenal glands and nearly 30% of the ovaries were found to be involved by metastases (Lumb and Mackenzie, 1959).

TABLE I.—*Metastases in* 348 *Patients*

Bone	212 (61%)	Brain	12 (3%)
Bone and viscera	172 (50%)	Heart	4 (1%)
Lung and pleura	108 (31%)	Local recurrences of primary tumour	90 (26%)
Liver	59 (17%)		
Eye	14 (4%)		

Choice of Patients for Adrenalectomy

Only half of those submitted to adrenalectomy are likely to benefit from it. The choice of patients for bilateral adrenalectomy therefore presents some difficulty, as there is today no simple, reliable, and generally applicable test to predetermine "hormone-dependence." The response to adrenalectomy is not solely determined by the biological factor of hormone-dependence, and other factors should be taken into consideration.

Extent, Type, and Site of Metastases

The extent of metastatic involvement and the interference with vital functions increase the operative hazard and influence the response to treatment.

The size of the metastases and the type of invasion and spread are more important than the site. Thus hepatic metastases have been reported as less likely to respond than skeletal metastases, but a study of patients with hepatic metastases indicates that a large number of small metastatic nodules may regress completely, whereas one or two large massive deposits will not be affected.

Similarly, pulmonary metastases revealed radiologically as multiple, discrete, spherical shadows may regress completely after adrenalectomy, whereas infiltration of the pulmonary lymphatics (lymphangitis carcinomatosa) invariably fails to respond. Similarly, intracranial metastases at the base of the skull or cerebral or cerebellar metastases may respond if small although multiple, whereas large tumours may kill the patient from a sudden haemorrhage and raised intracranial pressure. These observations indicate that adrenalectomy should be undertaken as soon as metastases are diagnosed and not as a last resort.

Confusion of thought between failure to respond, owing to hormone-independence of the cancer and functional failure of

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