THE URINARY METABOLITES OF TESTOSTERONE: AN INDEX OF TESTICULAR FUNCTION IN CHILDREN

by

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CONTENTS

PREFACE AND ACKNOWLEDGEMENTS

This thesis is necessarily lengthy to accommodate the innumerable results presented. I apologise for the voluminous nature of this work although the reader may note that this thesis contains many figures and tables throughout the text, whilst a further 81 pages of individual patient results (Chapter 5) are included for perusal by the interested reader.

The studies presented in this thesis were carried out by the author in the Steroid Laboratory of the University Department of Child Health, Royal Hospital for Sick Children, Glasgow. The investigation was supported by a grant to Dr William Hamilton from the New Medical Development Fund of the Scottish Home and Health Department.

These investigations were developed as a service to clinicians throughout Scotland and I am indebted to all those concerned for permission to study their patients. I am also grateful to Dr J. Williamson, Carlisle; Dr A. McCandless, Liverpool; Dr B. De Lobo, Luton; Dr G. Komrower, Manchester; Dr C. Cooper, Newcastle upon Tyne, England, and Professor W. Swoboda in Vienna, Austria, for the opportunity to study their patients.

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I remain grateful to Dr A. Gibson for histological preparation of tissues and Mr A. Herd (Department of Medical Illustration) for preparation of the photographs in Chapter 2. The patience of my wife in typing this volume exceeded my expectations.

Parts of this work have been presented at the first, second and third meetings of the British Paediatric Endocrine Group (Lancaster, April 1973; Harrogate, April 1974; Birmingham, November 1974).

COMPUTING AND STATISTICAL PROCEDURES

The results in this thesis have been analysed by the writer using the Statistical Package for the Social Sciences (SPSS) on the IBM 370 computer installation at the University of Newcastle upon Tyne.

The results presented and the published normal ranges used for comparison were routinely analysed for skewness and kurtosis. Skewed and kurtotic distributions were compared statistically following logarithmic transformation to a normal distribution. Although logarithmic transformation is shown graphically only for plasma testosterone (Figure 3.4, page 133) it should be noted that most of the individual urinary steroids results in the literature required transformation to a normal distribution before statistical analysis.

Statistical results following logarithmic transformation were similar to results obtained using nonparametric statistics alone.

S.I. UNITS

Since writing this thesis the Système International d'Unités (S.I. Units) has been adopted on DHSS instructions (HSC (IS) 140. **Metrication: Introduction into Medicine of the International System of Units).** The relevant factors for conversion of the results presented in this thesis to S.I. Units are given below.

Plasma testosterone	ng/100 ml x 0.0347	nM/l
Urinary creatinine	mg/24 hrs x 8.84	μM/24 hrs
Urinary steroids		
17-oxosteroids	mg/24 hrs x 3.47	μM/24 hrs
5α-androstane-3α,17β-diol 5β-androstane-3α,17β-diol 5α-androstane-3β,17β-diol	μg/24 hrs x 3.42	nM/24 hrs
Δ^5-androstene-3β,17β-diol androsterone aetiocholanolone epiandrosterone	μg/24 hrs x 3.44	nM/24 hrs
dehydroepiandrosterone testosterone	μg/24 hrs x 3.47	nM/24 hrs
11β-hydroxyandrosterone 11β-hydroxyaetiocholanolone	μg/24 hrs x 3.26	nM/24 hrs

STEROID NOMENCLATURE

To facilitate fluency, trivial names of steroids have been used where possible in this thesis. The trivial names and abbreviations used are listed below together with the approved systematic nomenclature of the IUPAC - IUB Commissions as described in Biochem. J. (1969) 113: 5.

TRIVIAL NAME	SYSTEMATIC NAME
Aetiocholanolone (Ae)	3α-Hydroxy-5β-androstan-17-one
5α-Androstanediol (5α-diol)	5α-Androstan-3α,17β-diol
5β-Androstanediol (5β-diol)	5β-Androstan-3α,17β-diol
5α-Androstanedione	5α-Androstan-3,17-dione
5β-Androstanedione	5β-Androstan-3,17-dione
Δ^4-Androstenediol	4-Androstene-3β,17β-diol
Δ^5-Androstenediol (Δ^5-diol)	5-Androstene-3β,17β-diol
Androstenedione	4-Androstene-3,17-dione
Androsterone (A)	3α-Hydroxy-5α-androstan-17-one
Androsterone glucuronoside	3α-Hydroxy-5α-androstan-17-one 3-glucuronoside
Androsterone heptafluorobutyrate	3α-Hydroxy-5α-androstan-17-one 3-heptafluorobutyrate
Androsterone sulphate	3α-Hydroxy-5α-androstan-17-one 3-sulphate
Cholesterol	5-Cholestene-3β-ol
Cortisol	11β,17α,21-Trihydroxy-4-pregnene-3,20-dione

Dehydroepiandrosterone (DHA)	3β-Hydroxy-5-androstene-17-one
Dehydroepiandrosterone glucuronoside	3β-Hydroxy-5-androstene-17-one 3-glucuronoside
Dehydroepiandrosterone sulphate	3β-Hydroxy-5-androstene-17-one 3-sulphate
5α-Dihydrotestosterone (DHT)	17β-Hydroxy-5α-androstan-3-one
5α-Dihydrotestosterone heptafluorobutyrate	17β-Hydroxy-5α-androstan-3-one 17-heptafluorobutyrate
5β-Dihydrotestosterone (β-DHT)	17β-Hydroxy-5β-androstan-3-one
5β-Dihydrotestosterone heptafluorobutyrate	17β-Hydroxy-5β-androstan-3-one 17-heptafluorobutyrate
Epiandrosterone (EpiA)	3β-Hydroxy-5α-androstan-17-one
Epiandrosterone glucuronoside	3β-Hydroxy-5α-androstan-17-one 3-glucuronoside
Epiandrosterone heptafluorobutyrate	3β-Hydroxy-5α-androstan-17-one 3-heptafluorobutyrate
Epiandrosterone sulphate	3β-Hydroxy-5α-androstan-17-one 3-sulphate
Epitestosterone	17α-Hydroxy-4-androstene-3-one
11β-Hydroxyaetiocholanolone (11β-OH-Ae)	3α,11β-Dihydroxy-5β-androstan-17-one
11β-Hydroxyandrosterone (11β-OH-A)	3α,11β-Dihydroxy-5α-androstan-17-one
20α-Hydroxycholesterol	5-Cholestene-3β,20α-diol
17α-Hydroxypregnenolone	3β,17α-Dihydroxy-5-pregnene-20-one
17α-Hydroxyprogesterone	17α-Hydroxy-4-pregnene-3,20-dione
11β-Hydroxytestosterone	11β,17β-Dihydroxy-4-androstene-3-one
6β-Hydroxytestosterone	6β,17β-Dihydroxy-4-androstene-3-one
17β-Oestradiol	1,3,5(10) Estratriene-3,17β-diol

TRIVIAL NAME	SYSTEMATIC NAME
Oestriol	1,3,5(10)-Estratriene-3,16α,17β-triol
Oestrone	3-Hydroxy-1,3,5(10)-estratriene-17-one
11-Oxoaetiocholanolone	3α-Hydroxy-5β-androstan-11,17-dione
11-Oxoandrosterone	3α-Hydroxy-5α-androstan-11,17-dione
Pregnanediol	5β-Pregnane-3α,20α-diol
Pregnanetriol	5β-Pregnane-3α,17α,20α-triol
Pregnenolone	3β-Hydroxy-5-pregnene-20-one
Progesterone	4-Pregnene-3,20-dione
Testosterone	17β-Hydroxy-4-androstene-3-one
Testosterone glucuronoside	17β-Hydroxy-4-androstene-3-one 17-glucuronoside
Testosterone mono-heptafluorobutyrate	17β-Hydroxy-4-androstene-3-one 17-heptafluorobutyrate
Testosterone di-heptafluorobutyrate	17β-Hydroxy-4-androstene-3-one 3,17-heptafluorobutyrate
Testosterone sulphate	17β-Hydroxy-4-androstene-3-one 17-sulphate

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Δ^5-3β-Hydroxysteroids	Steroids having a 3β-hydroxyl group and a double bond between C5 and C6.
Δ^4-3-Oxosteroids	Steroids having a 3-oxo group and a double bond between C4 and C5.

ABBREVIATIONS

The main abbreviations used in this thesis are listed below.

ACTH	Adrenocorticotrophic hormone
d.p.m.	Disintegrations per minute
ECD	Electron capture detection
EDTA	Ethylenediaminetetra-acetate
FID	Flame ionization detection
FSH	Follicle stimulating hormone
GLC	Gas liquid chromatography
HCG	Human chorionic gonadotrophin
hGH	Human growth hormone
ICSH	Interstitial cell stimulating hormone
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
NAD^+	Nicotinamide-adenine dinucleotide (oxidised form)
NADPH	Nicotinamide-adenine dinucleotide phosphate (reduced form)
p	Probability
r	Correlation coefficient
S.A.	Specific activity
TeBG	Testosterone-binding β-globulin
TLC	Thin layer chromatography

CHAPTER 1

INTRODUCTION

HISTORICAL REVIEW

"Let it not be supposed that the use of the hormones is a new discovery, far from it, it is as old as life itself ... As evidence that gland therapy is not new it is interesting to know that as far back as 600 BC orchitic substance was used as a remedy for obesity and orchitic extract was also extensively used by the ancient Egyptians and Romans for the purpose of rejuvenation and the perpetuation of youth."

C. Kempster, MRCS LRCP (1935)

In 1849 Berthold, studying testicular implants in capons, concluded that the testes possessed a dual function - the production of spermatozoa and the elaboration of an internal secretion. Forty years later, Brown-Sequard gave great impetus to the concept of internal secretion by the injection of testicular extract in his famous rejuvenation experiments. Although his work (Brown-Sequard, 1889) has been generally discredited, he did create great interest in the study of sex glands.

During the following thirty years, hypogonadism was treated by the administration of testicular extracts or by the transplantation of gonadal tissue. In 1891 Poehl, a Russian physiologist, claimed that spermine, an organic amine present in semen, was the active secretion of the testis. He treated numerous conditions ranging from scurvy to syphilis with spermine, apparently successfully. Although the pharmaceutical industry began the manufacture of spermine, Poehl's 'spermine theory' aroused much controversy, was subjected to severe criticism, and was finally totally rejected.

Bouin and Ancel (1903, 1904) first ascribed the role of hormone production to the Leydig cells. This was confirmed by numerous experiments on the selective destruction of the seminiferous tubules in animal testes by irradiation. Androgenicity remained unimpaired (Bergoine and Tribondeau,

1904; Regaud and Dubreuil, 1907) in the treated animals. Not everybody accepted that the testes were capable of hormone production. According to the older physiologists the effects of the testes were mediated through the nervous system (Nussbaum, 1906). However, the numerous results obtained by the transplantation of gonadal tissue into previously castrated animals proved Nussbaum to be wrong.

Many workers offered evidence for a secretion within the Leydig cells and in the neighbouring blood capillaries and lymphatics (Duesburg, 1918). This diffuse secretion was believed to be lipid in nature and the precursor of a true secretion (Mulon, 1910). These observations have been recently confirmed by Baillie (1964), who demonstrated the presence, in high concentration, of cholesterol in the Leydig cells of mature mice.

Research on the internal secretions was furthered by the work of Zondek (1926), Smith (1926) and Smith and Engle (1927). These authors reported undisputed evidence of the effect of the anterior pituitary on the growth and function of the gonads. Aschheim and Zondek (1928) subsequently found a substance in the urine of pregnant women which stimulated the gonads.

The discovery by McGee (1927) that a concentrated lipid extract of bull testis produced measurable comb-growth in the Leghorn capon within five days (McGee et al., 1928) laid the foundations for subsequent extraction procedures and for quantitative methods of assay.

After the isolation of oestrone from human pregnancy urine (Doisy et al., 1930) investigations turned to human urine for androgenic activity. Following the isolation of crude extracts possessing little androgenic activity (Loewe and Voss, 1930; Funk et al., 1930), rapid progress was made by Butenandt (1931, 1932) who isolated and subsequently characterized (1934) androsterone and dehydroepiandrosterone (Butenandt and Dannenbaum, 1934) from human urines. Butenandt believed he had isolated the male hormone in androsterone. However, it was found that its effects differed in many respects from those produced by testicular extracts.

Gallagher and Koch (1934a, 1934b) had shown that extracts of testicular tissue could be purified to yield a concentrate 6-10 fold more active than androsterone. The potency of this extract decreased when boiled with alkali. Instability to alkali was not characteristic of any of the known urinary androgens but was known to be exhibited by α, β-unsaturated ketones (progesterone). David et al. (1935) subsequently isolated from bull testis extracts pure testosterone in crystalline form.

Shortly after the relation of androsterone to cholesterol had been established (Ruzicka et al., 1934) many related compounds were prepared and their androgenic activities determined. The search for new compounds more potent than androsterone was on. In the decade following the synthesis of testosterone from cholesterol by Butenandt and Hanisch (1935) and Ruzicka et al. (1935) these two research groups, working in co-operation with Schering in Berlin and Ciba in Basel respectively, prepared and assayed numerous analogues of testosterone. The only compounds found with androgenic activity comparable to that of testosterone were 5α-dihydrotestosterone and the methyl esters of testosterone and 5α-dihydrotestosterone.

The advent of the Zimmermann reaction (Zimmermann, 1935) for ketones marked the first chemical assay in the study of endocrinology. The recognition of 17-ketosteroids as the quantitatively most important group of steroids giving this reaction gave impetus to numerous investigations attempting to correlate the urinary excretion of neutral 17-ketosteroids with clinical features of androgen action. Modifications of this method have been proposed (Callow et al., 1938; Holtorff and Koch, 1940) and these were reviewed by Zimmermann (1955). Although testicular androgens and their metabolites contribute to the 'Zimmermann chromogens', approximately two-thirds of the urinary excretion of neutral 17-ketosteroids are now known to derive from dehydroepiandrosterone and corticosteroid metabolites of adrenal origin (Callow, 1939). The estimation of urinary neutral

17-ketosteroids has, however, been standardized (17-oxosteroids; Medical Research Council, 1963) and the assay has been retained for routine urinary steroid analyses.

It was soon realised that more information could be obtained from the separation of the neutral 17-ketosteroids either into groups with common characteristics or into individual compounds. The most simple fractionation described separates the neutral 17-ketosteroids into the 3α-hydroxy and 3β-hydroxy fractions (α- and β-fractions) by the precipitation of the 3β-hydroxy-17-ketosteroids with digitonin (Butt et al., 1948; Haslam and Klyne, 1952).

The differential separation of neutral 17-ketosteroids was first described by Dingemanse et al. (1946, 1952). This method utilizes acid hydrolysis of the urine with subsequent separation of the neutral 17-ketosteroids by aluminium oxide adsorption chromatography, into eight individual fractions, each subsequently quantitated by the Zimmermann reaction. This method has been modified many times without successfully overcoming the inherent difficulties (Dorfman, 1968). However, the paper chromatographic separation of individual 17-ketosteroids introduced by Rubin et al. (1953) and the gradient elution methods used by Lakshmanan and Lieberman (1954) and Kellie and Wade (1957) gave more satisfactory results.

Numerous attempts to determine testosterone in biological samples have been reported, but only in the last 14 years have techniques been available for the estimation of testosterone in plasma and urine. Circulating testosterone was first isolated and identified in spermatic vein blood of the dog (West et al., 1952) and later in human spermatic vein blood (Lucas et al., 1957; Hollander and Hollander, 1958).

The work of Hollander and Hollander (1958) had shown that human testicular blood contained between 3 and 160 μg testosterone per 100 ml.

No testosterone was, however, detected in the peripheral venous blood of the subjects investigated. Oertel and Eik-Nes (1959) confirmed this finding but they succeeded in isolating testosterone in systemic blood of the normal male after the administration of large doses of human chorionic gonadotrophin (HCG). In 1961 Finklestein et al. were the first to describe a method for the estimation of testosterone in peripheral venous plasma. By their method testosterone was enzymatically converted to 17β-oestradiol and measured thereafter fluorimetrically. Although this method required large amounts of blood for adequate assay, it confirmed the presence of testosterone in the peripheral circulation of normal men and women. Sensitive methods for the estimation of testosterone in small amounts of human plasma were subsequently reported (Riondel et al., 1963; Hudson et al., 1963; Brownie et al., 1964; van der Molen et al., 1966). More recent methods applicable to the quantitation of plasma testosterone in prepubertal children will be reviewed in Table 3.VIII (Chapter 3).

Numerous but unsuccessful attempts were made to demonstrate testosterone in the urine. In 1950, Dobriner and Lieberman reported that administration of large amounts of testosterone to normal subjects resulted in the excretion in the urine of only trace amounts of the hormone. Ten years later, however, Schubert and Wehrberger (1960) demonstrated that testosterone is an excretory product in man. Values for the urinary excretion of testosterone have been reported for humans in health and disease (Camacho and Migeon, 1963, 1964). Their method employed hydrolysis with a commercial preparation of β-glucuronidase. Thus testosterone is present as the glucuronoside. Subsequent work has shown that testosterone may also be excreted 'free' (unconjugated) (Dulmanis et al., 1964) and as a sulphate (Dessypris et al., 1966). Again, more recently published methods applicable to the quantitation of urinary testosterone excretion in prepubertal children will be reviewed in Table 3.V (Chapter 3).

STEROID BIOSYNTHESIS IN THE TESTIS

Although this thesis is primarily concerned with the metabolism of testosterone it is perhaps pertinent to review briefly the biosynthesis of testosterone within the testis.

The interstitial cells of the testis were first described by Leydig in 1850. He noted these cells as clear, round cells, analogous to embryonic connective tissue, vacuolated and containing pigment granules and fat droplets Mulon (1910) believed these fat droplets to increase during secretory activity and envisaged this lipoidal substance to be the precursor of the true testicular secretion.

Baillie (1964) reported the presence of high concentrations of cholestero in Leydig cells of mature mice, whilst Christensen (1965) correlated the cholesterol production in the testis with the amount of agranular reticulum in the Leydig cells. Further work by Christensen and Fawcett (1966) suggested that cholesterol is stored in the agranular reticulum *en route* to the mitochondria of these cells where it will be converted to pregnenolone.

Experiments *in vivo* (Mason and Samuels, 1961) and *in vitro* (Hall and Eik-Nes, 1962) have demonstrated that cholesterol is formed from acetate in the testes. Intermediates in the testicular formation of cholesterol have been identified (Tsai *et al.*, 1964; Salokangas *et al*, 1964, 1965) which strongly support the view that cholesterol formation within the testes is similar to that in the liver.

The total concentration of cholesterol in the testes is relatively high, but as a considerable amount of this testicular cholesterol is associated with the germinal epithelium, the pool of cholesterol involved in the biosynthesis of androgens is small (Hall, 1963). Although the high concentration of germinal cells in the testes and the low solubility of cholesterol in aqueous phases confuse the role of cholesterol as a precursor of testicular androgens (Hall, 1963), recent experiments indicate that

cholesterol is the physiological source of all testicular steroids (Hall, 1970).

The side chain cleavage of cholesterol to yield pregnenolone and isocapraldehyde takes place in the mitochondria within the Leydig cells (Toren et al., 1964). Halkerston et al. (1961) have shown that this cleavage requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen and they conclude that hydroxylation of the side chain of cholesterol occurs before scission. 20α-Hydroxy-cholesterol (Solomon et al., 1956) and 20α,22β-dihydroxy-cholesterol (Shimizu et al., 1962) have been suggested as hydroxylated intermediates for this reaction.

Hall and Young (1968), accepting this proposed pathway, examined the influence of interstitial cell stimulating hormone (ICSH) on the conversion of 20α-hydroxy-cholesterol-^{3}H to testosterone-^{3}H in the testes. They found ICSH did not increase testosterone formation and since the point of trophic hormone action occurs later than the cholesterol side chain cleavage step (Koritz and Hall, 1965), they concluded that the 20α-hydroxylase system is specifically stimulated by ICSH. This confirmed the earlier suggestion of Halkerston et al. (1961) and Koritz (1962).

De novo synthesis of testosterone from acetate and cholesterol has been established in the human testis by both in vitro and in vivo methods (Brady, 1951; Savard et al., 1952; Rice et al., 1966; Knapstein et al., 1968; Serra et al., 1970). Although these authors have isolated many of the intermediates there has been no systematic elucidation of the preferred pathway from Δ^{5}-pregnenolone to testosterone.

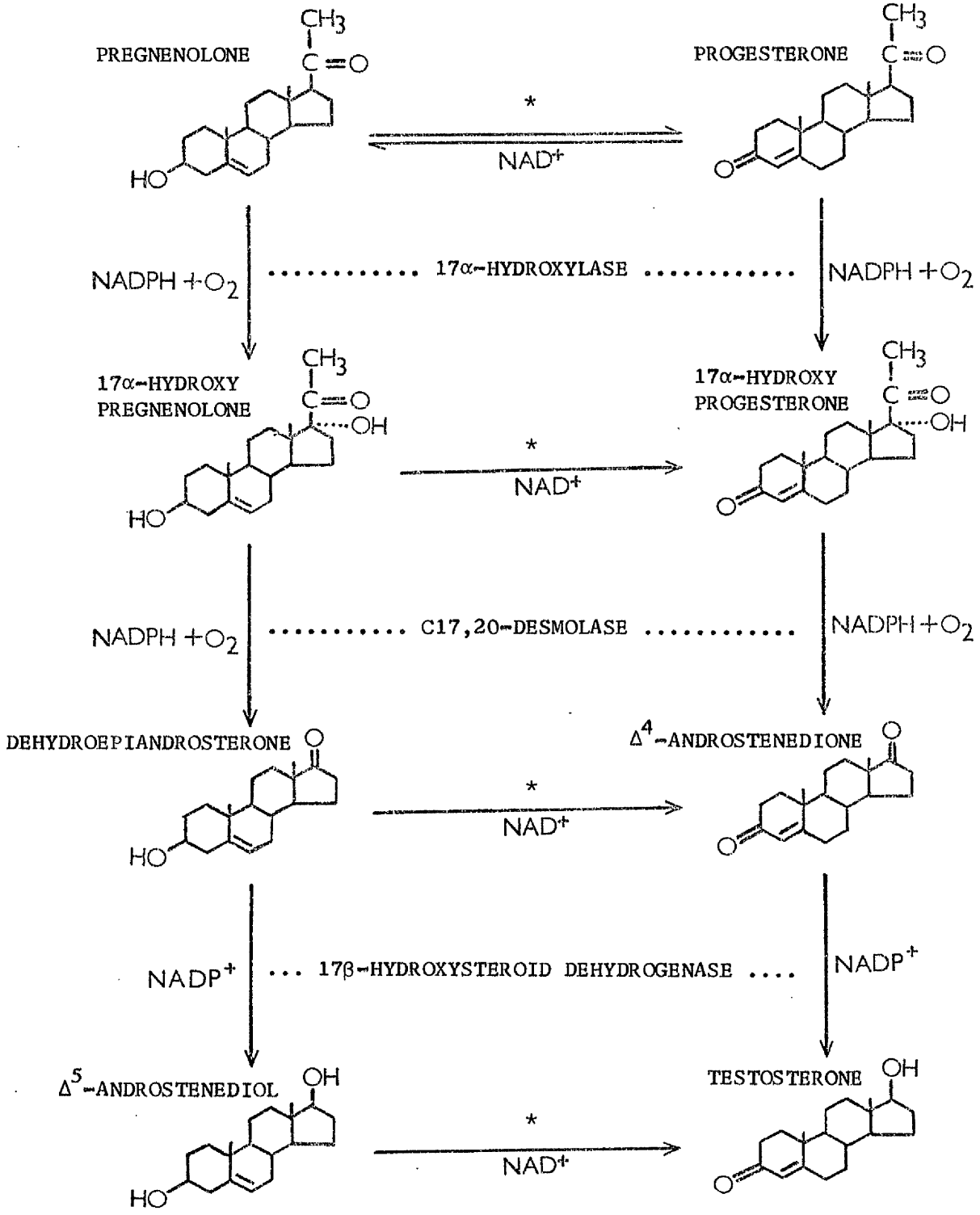
In 1937, Koch presented a theoretical scheme for the biosynthesis of testosterone and oestrogens. He suggested that cholesterol was converted via dehydroepiandrosterone and Δ^{5}-androstene-3β,17β-diol to testosterone. Koch also suggested that dehydroepiandrosterone was oxidized to Δ^{4}-androstenedione and the possibility that Δ^{4}-androstenedione and testosterone were aromatized to form oestrogens.

The Δ^4-pathway for testosterone biosynthesis (progesterone, 17α-hydroxyprogesterone, Δ^4-androstenedione) in the testis was initially demonstrated in the rat (Slaunwhite and Samuels, 1956) and in the human (Slaunwhite et al., 1962a) by studies using radioactively labelled Δ^4-steroid precursors of testosterone. Subsequently, the Δ^5-pathway (Δ^5-pregnenolone, 17α-hydroxypregnenolone, dehydroepiandrosterone, Δ^5-androstene-3β,17β-diol) was demonstrated in normal (Axelrod, 1965; Rosner and Macome, 1970) and abnormal (Gual et al., 1962; Charreau and Villee, 1968) human testes. However, it should be noted that these two predominant pathways may overlap since in vivo experiments on the canine testis (Hagen and Eik-Nes, 1964) demonstrate that 17α-hydroxypregnenolone can be converted to 17α-hydroxyprogesterone. Rosner et al. (1965a) have found that progesterone can be converted to Δ^5-pregnenolone by sliced rabbit testis in vitro although the physiological significance of this reverse reaction remains obscure.

Regardless of the pathway, five enzymes are required for the synthesis of testosterone from Δ^5-pregnenolone (Figure 1.1). All these enzymes, 17α-hydroxylase, 17,20-desmolase, 17β-reductase, 3β-hydroxysteroid dehydrogenase and isomerase, are contained in the microsomal fraction within the Leydig cells (Shikita and Tamaoki, 1965).

FACTORS AFFECTING ANDROGEN PRODUCTION

It has been reported (Acevedo et al., 1961, 1963; Bloch, 1964; Bloch et al., 1962; Ikonen and Niemi, 1966; Hamilton, 1971a) that human foetal testes are able to convert Δ^5-pregnenolone, progesterone and other precursors to testosterone. Lipsett and Tuller (1965) have demonstrated that the conversion of Δ^5-pregnenolone to testosterone increased markedly just before and during differentiation of the rabbit foetal Wolffian duct derivatives and they correlated this differentiation of the male reproductive system with the appearance of the Leydig cells.



* = Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE/ISOMERASE COMPLEX

Figure 1.1 Testosterone biosynthesis in the testis from Δ^5-pregnenolone

Lindner (1961) studied testosterone and androstenedione concentrations in bovine spermatic vein blood. His important observation that the testosterone concentration rose sharply at puberty and the correlation of this rise with the first production of fructose by the seminal vesicles marked the first true assay of testicular function.

Snipes et al. (1965) found that in vitro incubation of testicular tissue from younger guinea pigs produced more androstenedione but less testosterone from progesterone and concluded that the activity of 17β-dehydrogenase increased with age until full maturation. Nayfeh et al. (1966) showed that in immature rats the testosterone synthesized from progesterone by testicular tissue was further metabolized to 5α-androstane-3α,17β-diol. These authors suggested a specific function for this metabolite in sexual maturation. Hamilton et al. (1970) stressed the importance of the Δ^5-3β-hydroxysteroid pathway in early foetal life.

When animals were treated with gonadotrophin (HCG, LH and FSH), the overall synthesis of testosterone from acetate was enhanced (Hall and Eik-Nes, 1962; Hall, 1963; Eik-Nes and Hall, 1965). Gonadotrophin has been reported to stimulate specifically the action of Δ^5-3β-hydroxysteroid dehydrogenase (Samuels and Helmreich, 1956) and 20α-hydroxylase (Hall and Young, 1968).

X-irradiation of the testis causes complete destruction of spermatogenesis without appreciable histological damage to the interstitial cells. However, it has been reported that X-irradiated testes have reduced biosynthetic potential due to the reduced activity of 17α-hydroxylase and 17,20-desmolase (Schoen, 1964). Recently the activity of 17β-dehydrogenase has also been reported to be reduced after X-irradiation of the testes (Inano and Tamaoki, 1968b). As 17β-dehydrogenase does not require NADPH as a co-factor it would appear that the action of X-irradiation of the testis is not on the NADPH generating system within the Leydig cell as previously suggested by Berliner et al. (1964).

METABOLISM OF TESTOSTERONE

The principal site of steroid catabolism is the liver. The hepatic metabolism of androgens results in the formation of relatively nonandrogenic steroids conjugated with sulphuryl or glucuronyl residues. This hepatic metabolism is mainly reductive and consists of the reduction of the double bond to form dihydrosteroids, the reduction of the 3-oxo-dihydrosteroids to form the corresponding 17-oxosteroids and the conjugation of these 17-oxostero: or the further metabolized androstanediols, with sulphuryl or glucuronyl residues forming the corresponding sulphates and glucuronides (Heftmann, 1970).

Although only small amounts of androgenic steroids are metabolized outside the liver, this extra-hepatic metabolism may represent an important aspect of the action of such steroids. It is now generally accepted that testosterone action is mediated through several closely related steroids in androgen dependent tissues. Bruchovsky and Wilson (1968) have shown that 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) is the major metabolite of testosterone in androgen dependent tissues both in vivo and in vitro. These authors located NADPH dependent 5α-reductase in the nuclear and cytoplasmic fractions, postulated selective nuclear binding of 5α-dihydrotestosterone, and demonstrated subsequent metabolism within the cytoplasm to 5α-androstanediol.

Unhjem and Tveter (1969) first isolated a high affinity androgen-binding protein in the prostate cytosol fraction. Fang and Liao (1971) were able to separate two soluble androgen binding proteins in the prostate cytosol one of which is highly specific for 5α-dihydrotestosterone. These authors postulated that 5α-dihydrotestosterone was selectively bound to a cytoplasmic receptor protein, this steroid-receptor complex is then transferred to the nucleus of the prostatic cells where it binds to acidic proteins of nuclear chromatin. Nuclear acceptor sites for 5α-dihydrotestosterone-receptor complexes were subsequently reported by O'Malley (1971). The entry of

5α-dihydrotestosterone into the prostatic cell nucleus is believed to facilitate RNA synthesis, protein synthesis and androgen directed growth (Williams-Ashman and Reddi, 1971).

Baulieu et al. (1968) studied the prostatic effects of testosterone metabolism and concluded that 5α-dihydrotestosterone caused cell division and eventual hyperplasia of the tissues whilst 5α-androstane-3β,17β-diol caused hypertrophy and secretion. Later Baulieu (1970) in a review of the 'endocrinology of metabolites' concluded that as testosterone action within the prostate can be explained by the discrete actions of testosterone metabolites, testosterone may act as a prehormone, rather than a hormone in certain target organs. This diversity of testosterone action may then explain the different activities of testosterone in target tissues.

Much of the work on testosterone metabolism was carried out by studying urinary metabolites. Early investigations demonstrated that when large amounts of testosterone were administered to humans there was an increase in 'bioassayable' androgen in the urine. The classic work of Callow (1939) showed that the main urinary metabolites of testosterone in the human were the conjugates of androsterone and aetiocholanolone. Dorfman (1941) reported the isolation of epiandrosterone from the urine of a hypogonadal male who had been given testosterone propionate. Further work by West et al. (1951) showed that the main metabolites of testosterone were excreted as the glucuronosides.

With the advent of radioactively labelled testosterone, urinary studies on the metabolites of testosterone were simplified. Both ^{14}C-testosterone and ^{3}H-testosterone were used in early investigations (Gallagher et al., 1951; Fukushima et al., 1954) and similar results were obtained with both isotopes. These workers confirmed that androsterone and aetiocholanolone were the main metabolites but in addition they isolated 5α-androstanedione and 5β-androstanedione as intermediates.

5α-Androstane-3α,17β-diol and 5β-androstane-3α,17β-diol, minor metabolites of testosterone, were believed to be reduction products of androsterone and aetiocholanolone respectively (Schiller et al., 1945) and it was presumed that the pathway to the androstanediols passed through androstenedione and the corresponding 17-oxosteroids. Baulieu and Mauvais-Jarvis (1964) have, however, suggested a direct '17β-hydroxyl pathway' from studies using [4-^{14}C,17α-^{3}H]testosterone (Figure 1.2).

Other minor metabolites of testosterone have been isolated from urine (Δ^{16}-androstene-3α-ol, Bulbrook et al., 1963; 6β-hydroxytestosterone and 11β-hydroxytestosterone, Schubert et al., 1964) although the physiological significance of such metabolites remains obscure.

The metabolism of radioactively labelled testosterone glucuronoside has been studied by Robel et al. (1966a, 1966b). These workers found only 5β-metabolites (aetiocholanolone and 5β-androstane-3α,17β-diol) and postulated a 'direct' 5β-metabolism of testosterone glucuronoside, showing that this conjugate is not merely an end-product of steroid metabolism.

The work of Vande Wiele et al. (1963), Korenman et al. (1964), Baulieu and Mauvais-Jarvis (1964), Rivarola et al. (1966), Horton and Tait (1966) and Baulieu (1967) on the transformation of circulating Δ^{5}-androstenediol, androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulphate to testosterone glucuronoside showed that some of the testosterone is formed in the liver from these precursors. Most of this hepatic testosterone does not leave the liver, but is extensively catabolized in situ, particularly to testosterone glucuronoside, this then being excreted in the urine. The apparent 'compartmentalization' of testosterone metabolism and the mathematical analyses performed on such investigations have been reviewed by Baulieu (1967). Such studies have demonstrated that the same steroid hormone may be differently metabolised depending on the precursor from which it is derived, its place of formation, and its state of conjugation.

Figure 1.2 The principal metabolic pathways from testosterone

The following metabolites are represented:

1 Δ^4-androstenedione

2 5α-androstanedione

3 5β-androstanedione

4 androsterone

5 epiandrosterone

6 aetiocholanolone

7 5β-androstan-3β ol-17 one

8 5α-androstane-3α,17β-diol (5α-diol)

9 5α-androstane-3β,17β-diol (3β-diol)

10 5β-androstane-3α,17β-diol (5β-diol)

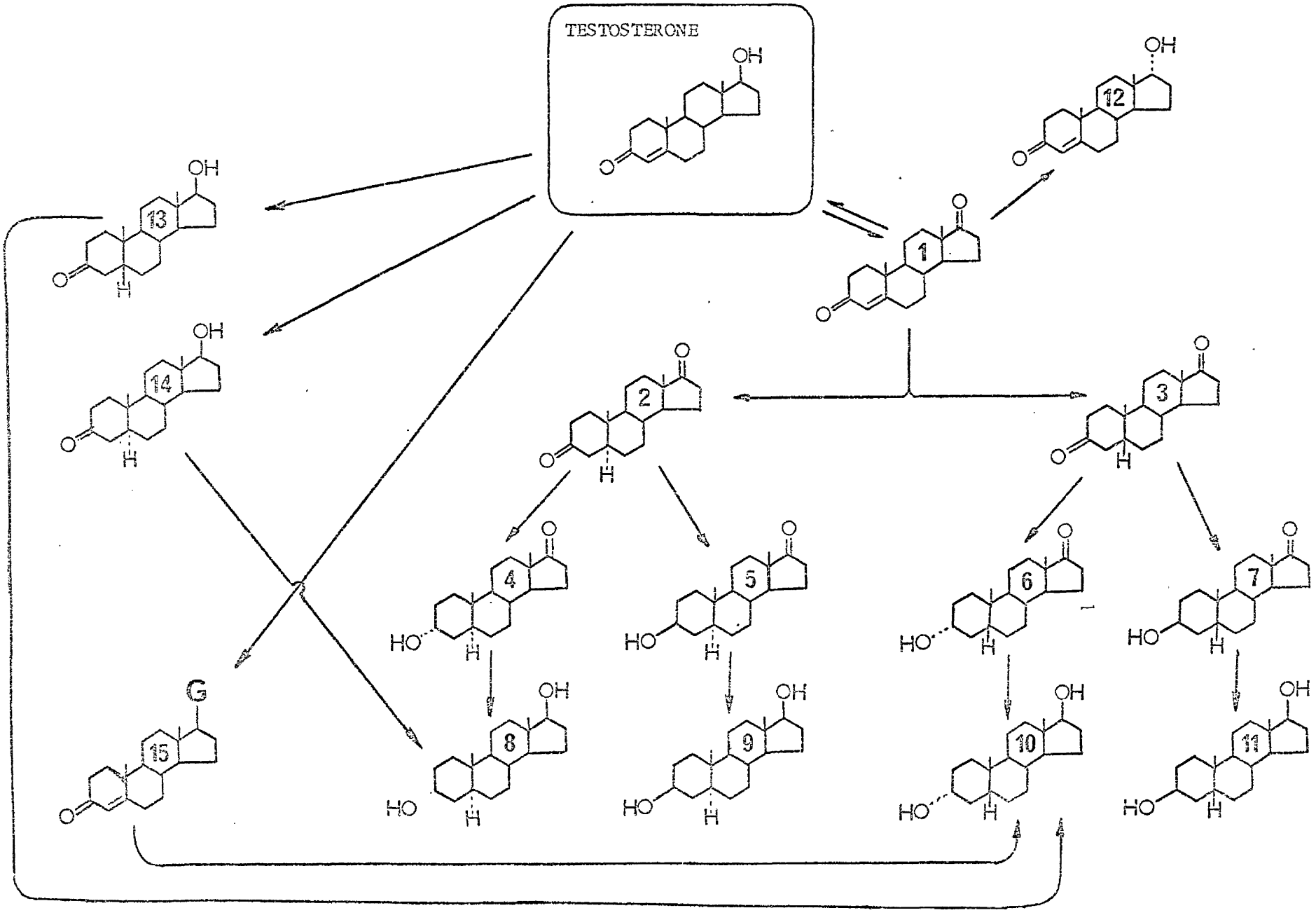
11 5β-androstane-3β,17β-diol

12 epitestosterone

13 5β-dihydrotestosterone (5β-DHT)

14 5α-dihydrotestosterone (5α-DHT)

15 testosterone glucuronoside


TESTOSTERONE
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Nearly all the catabolites of testosterone are ultimately excreted in the urine. However, the urinary excretion rates of many of the androgen metabolites may not reflect their production rates since they may be exposed to further catabolism (Baulieu et al., 1965; Robel et al., 1966a).

Study of the urinary androgen metabolites remains of clinical diagnostic significance. Accepting that testosterone is not the only source of such metabolites and these therefore reflect a composite endocrinological spectrum of testicular, adrenal, hepatic and peripheral metabolism, the study of urinary androgen metabolites may still give information on the actual utilization of testosterone within the patient.

In the last decade many methods which estimate the majority of urinary steroid metabolites important for differential endocrinological diagnosis have been published. Most of these methods utilize gas-liquid chromatography as the only chromatographic separation (Haahti et al., 1961; Sparagana et al., 1963; De Paoli et al., 1963; Ruchelman and Cole, 1966). The profiling of urinary steroids first introduced by Gardiner and Horning in 1966 has been of diagnostic use in many types of endocrinological disturbances (Van Kampen and Hoek, 1967; Hoek and Van Kampen, 1968). Recent methods, however, have the added advantage of allowing calculation of the rates of excretion of individual steroids from such profiles (Horning et al., 1969; Garmendia et al., 1971).

NORMAL SEXUAL DIFFERENTIATION IN THE MALE FOETUS

The development of the male phenotype and of normal testicular function is dependent on several determinating processes exerted from the time of ovum fertilization. A very brief review of such factors is pertinent to the aetiology of the pathological conditions later to be considered in this thesis

The gonads develop from primitive germ cells of extragonadal origin (Witschi, 1948), coelomic (germinal) epithelial cells, and cells of the mesonephric mesenchyme. The gonadal ridges develop about the fourth week of

foetal life and appear as elongated ridges on the ventral surface of the mesonephros. By the sixth week of gestation germ cells can be recognised in the now rounded gonad. At this indifferent stage of development, the gonad consists of an outer cortex (potentially the definitive ovary) and an inner medulla (potentially the definitive testis).

Witschi (1951) postulated a dual system of intragonadal inductors released from the medulla and cortex of the amphibian gonad. Under the influence of the male sex chromosomes these inductor substances from the rudimentary sex cords suppress cortical development and favour differentiation of the medulla into a testis. Burns (1955) believed these inductor substances to be steroids, similar to the androgens and oestrogens, although Witschi (1965) does not accept that they are steroidal in nature. Jost (1970) has recently postulated a mammalian masculinization factor released under the influence of the Y chromosome, whilst Mittwoch (1970) reported that testicular differentiation of the indifferent gonad may be caused by the differential growth rates of the two components.

At this indifferent stage a double set of sexual ducts are present, the male (Wolffian) ducts and the female (Müllerian) ducts, together with the bipotential primordia of the external genitalia. On testicular differentiation, the Müllerian ducts regress whilst the Wolffian ducts undergo further growth and differentiation, thus forming the epididymis, vas deferens, seminal vesicles and ejaculatory duct. The development of the internal and external genitalia correlates with the appearance of the Leydig cells (Table 1.I).

Since the observations of Bouin and Ancel (1904) of testicular secretion in pig embryos and their correlation of this with masculine-orientated organogenesis, many investigations of the role of the gonadal hormones in the differentiation of the internal and external genitalia have been performed using animal experiment. The classical *in vivo* studies on rabbit foetuses

TABLE 1.I CHRONOLOGY OF DIFFERENTIATION OF THE TESTES AND THE INTERNAL AND EXTERNAL GENITALIA IN THE MALE FOETUS

Weeks Gestation	Gonadal Characteristics	Wolffian Duct	Müllerian Duct	External Genitalia
3	Migration of germ cells	-	-	-
4	Indifferent gonadal ridges	-	-	-
6	Germ cells Seminiferous tubules	Present	Present	Undifferentiated
7	Leydig cells proliferating	Differentiating	Regressing	Penile urethra developing
12	Leydig cells maximum	Differentiation complete	Regressed	Development complete
18	Leydig cells regressing	Differentiation complete	Regressed	Development complete. Testis descending into scrotum
30	Leydig cells not visible	Differentiated	Regressed	Testes descended
40				

by Jost (1947, 1953, 1970) have shown that castration *in utero* after the gonads were histologically sexually differentiated, but before differentiation of the internal and external genitalia, produced feminization of the genital tract regardless of the genetic sex. Both *in vivo* (Jost, 1955) and *in vitro* (Picon, 1969) grafts of testicular tissue into castrated foetuses produced masculinization.

The observation that androgens, such as testosterone, can act in place of foetal testes in their masculinizing effects, although exogenous androgens fail to inhibit the Müllerian ducts (Jost, 1953), suggests that the testis produces a Müllerian duct inhibiting factor in addition to a masculinizing hormone. Further work with the potent antiandrogen, cyproterone acetate, in rabbit foetuses has confirmed this hypothesis (Elger, 1966; Jost, 1967; Neumann *et al.*, 1969). More recent work by Josso (1971, 1972) using explants of foetal rat reproductive tracts in organ culture with human foetal testes has shown the interspecific character of the Müllerian duct inhibiting factor and shown that this is not a 'free' steroid.

Recent animal experimentation has further shown that testosterone secreted by the foetal testes induces male differentiation of the urogenital tract by two different mechanisms. Testosterone itself is responsible for initiation of differentiation of the Wolffian duct into the epididymis, vas deferens and seminal vesicle, whilst 5α-dihydrotestosterone is responsible for the development of the urogenital sinus, genital tubercle and labioscrotal swellings into the prostate, penis and scrotum (Wilson and Lasnitzki, 1971).

Failure of the foetal testes to produce testosterone during differentiation of the external genitalia results in varying degrees of hypospadias to a complete lack of fusion of the labioscrotal folds. Foetal testicular insufficiency, in which there is a secretory failure of both testosterone and the Müllerian duct inhibiting factor, results in a

phenotypic female though infertile, whilst failure of testosterone production alone results in the formation of female external genitalia but with a blind ending vagina. The degree of ambiguity of the external genitalia, the degree of development of the Wolffian duct structures and the extent to which Müllerian elements persist depends on testicular function, the stage of development at which the defect occurs and the sensitivity of the end-organ tissues to these stimuli.

TESTICULAR FUNCTION IN MALE HYPOGONADISM

In the remaining part of this introduction I will discuss patients with abnormal sexual development and attempt to describe the underlying biochemical dysfunction.

Most classifications of male hypogonadism separate hypogonadism caused by primary testicular failure and hypogonadism secondary to a deficiency of pituitary gonadotrophin secretion. This subdivision will be used here (Table 1.II).

PRIMARY TESTICULAR FAILURE

Primary testicular failure can be subdivided into three groups: those of genetic origin, those due to developmental defects and those due to failures of spermatogenesis but with apparent normal Leydig cell function.

Klinefelter's Syndrome

In 1942 Klinefelter, Reifenstein and Albright described the syndrome characterized by small testes, azoospermia, normal external genitalia, gynaecomastia and a high urinary excretion of gonadotrophin. Although their paper first grouped such symptoms as a definite clinical entity, case reports of similar patients had been published in the early 19th century. In the original publication (Klinefelter *et al.*, 1942), gynaecomastia was considered a necessary symptom, whilst Heller and Nelson (1945) described patients without gynaecomastia but with the other characteristics of the syndrome. Becker *et al.* (1966) reported that approximately one-third of such patients have behavioural problems whilst 4 per cent are mentally retarde

TABLE 1.II CLASSIFICATION OF MALE HYPOGONADISM

Primary Testicular Failure			Pituitary Gonadotrophin Failure	
Genetic	Developmental	Seminiferous Tubules	Congenital	Pituitary Disease
(1) Klinefelter's syndrome	(1) Anorchia	(1) Mumps orchitis	(1) Hypogonadotrophic hypogonadism	(1) Lesions of pituitary or hypothalamus
(2) XX-male syndrome	(2) Cryptorchidism	(2) Irradiation	(2) Fertile eunuch syndrome	
(3) Noonan's syndrome	(3) Germinal cell aplasia	(3) Other causes	(3) Associated with congenital disorders	
(4) Associated with hereditary or inherited disorders				

In 1956, Bradbury et al., Riis et al., and Plunket and Barr almost simultaneously described a positive sex chromatin pattern (ie Barr body present) in many patients with Klinefelter's syndrome. Jacobs and Strong (1959) subsequently reported the chromosomal constitution 47,XXY in such patients. Not all apparent cases of Klinefelter's syndrome are, however, chromatin-positive. Approximately 25 per cent of cases reported in the literature are chromatin-negative (Barr, 1966) and it is possible that many of such cases may have mosaicism. Paulsen et al. (1968) described four such chromatin-negative cases of the syndrome, all of whom had an XY karyotype as judged from skin study. One case had an XY/XXY mosaic in blood and testis, whilst the other three cases had XY in the blood and XY/XXY in the testis alone. Thus the somatic features of Klinefelter's syndrome can be caused by an XXY stem-line in the testis.

Although the true clinical picture only develops at puberty, prepubertal patients have been described (Bunge and Bradbury, 1957; Tanner et al., 1959; Ferguson-Smith, 1959; Hamilton, 1971b) in patients with mental retardation, micro-orchidism or skeletal disorders.

No adequate explanation for the histological changes in the testes has yet been reported, although the testes fail to respond normally to pituitary follicle stimulating hormone (FSH). During the initial stages of puberty, the testes are characterized by the development of fibrous tissue, and only Sertoli cells in the seminiferous tubules. Although the proliferation of the fibrotic tissue may result in complete hyalinization of the tubules during adolescence, a wide range of pathological changes has been reported in patients with this syndrome. These range from normal spermatogenesis (Bunge and Bradbury, 1956) to complete atrophy of the seminiferous tubules. The Leydig cells characteristically show adematous clumping although the actual numbers may not be increased. Burt et al. (1954 using histochemical techniques for lipoidal substances, reported decreased function of the Leydig cells.

Slaunwhite et al. (1962b), studying in vitro incubation of testicular tissue from patients with Klinefelter's syndrome, found normal testosterone biosynthesis and concluded that such patients (XXY karyotype, atrophic testes, hyalinized seminiferous tubules with azoospermia and abundant Leydig cells) may possess a modified target organ insensitivity to testosterone. The defect in such patients has been ascribed to a decreased biosynthesis of testosterone (Gabrilove et al., 1963; Gabrilove, 1964) although recently an increased conversion to oestrogens has been suggested (Gabrilove et al., 1970) and confirmed by Sharma and Gabrilove (1971). Hamilton (1971b) has recently confirmed normal testosterone biosynthesis in younger patients with Klinefelter's syndrome but found a progressive failure with age.

Most of the indices of testosterone secretion have been found to be reduced in Klinefelter's syndrome. Low levels of testosterone in peripheral plasma (Lipsett et al., 1966; Hudson et al., 1967; Paulsen et al., 1968) and in testicular venous plasma (Dupré et al., 1964; Jeffcoate et al., 1967) have been reported. Testosterone production rates are also reduced (Lipsett et al., 1966; Hudson et al., 1967; Jeffcoate et al., 1967).

Several studies have suggested that the Leydig cells are maximally stimulated by endogenous gonadotrophin and respond poorly to administered HCG as measured by plasma testosterone (Lipsett et al., 1966; Hudson et al., 1967) whilst Paulsen (1968) reported normal, although limited, response in plasma testosterone following HCG administration.

The neutral 17-oxosteroids have been extensively studied in Klinefelter's syndrome and generally they are within (Stewart et al., 1959) or below (Giorgi and Sommerville, 1963) the low normal range of excretion. Johnsen (1956), from fractionation studies of the urinary 17-oxosteroids, reported very low aetiocholanolone excretion in such patients whilst Gilbert-Dreyfus et al. (1956) found a relative increase in the 3α-hydroxy-17-oxosteroids.

Recent work by Garmendia et al. (1971) using a GLC method for the separation and quantitation of individual neutral 17-oxosteroids has confirmed these older fractionation studies. They found elevated androsterone, decreased aetiocholanolone and decreased dehydroepiandrosterone excretion in two patients with Klinefelter's syndrome.

XX-Male Syndrome

By definition these patients have a male phenotype, male psychosexual identification, testes, absence of ovarian tissue, and absence of female genital organs. De la Chapelle et al. (1964) described the first male patient with the XX chromosome karyotype and no ambiguity of the external genitalia. Subsequent reports by Therkelsen (1964), de la Chapelle et al. (1965), Lindsten et al. (1966), de Grouchy et al. (1967), Sebaoun et al. (1969) and Bergman et al. (1970) have shown that this condition is a definite clinical entity. Clinically, XX-males resemble patients with Klinefelter's syndrome in psychosexual orientation, intelligence, secondary sex characteristics, testicular histology and hormonal status, the main difference being their height. XX-males are much smaller than patients with Klinefelter's syndrome, smaller than normal males but taller than normal females.

Although the penis and scrotum are generally of normal size in such patients, testicular size is invariably decreased, and their consistency ranges from firm (in young patients) to soft (mostly in older patients). Gynaecomastia has been reported in two such cases (Strauch et al., 1965; Sebaoun et al., 1969). Histologically the testes generally show absence of spermatogonia, diminished tubular diameter, hyalinization and obliteration of the tubules and Leydig cell hyperplasia.

Sebaoun et al. (1969) have reported hormone studies in a 15-year-old XX-male. They found diminished excretion of total urinary 17-oxosteroids, diminished excretion of urinary androsterone and aetiocholanolone, but

normal excretion of urinary dehydroepiandrosterone and the 11β-hydroxy- and 11-oxo-17-oxosteroids, and concluded that there was normal adrenal function but poor testicular function. Ismail et al. (1968) reported an abnormally low excretion of urinary testosterone in three adult patients with this syndrome.

Noonan's Syndrome

In 1930 Ullrich reported an 8-year-old girl with congenital lymphedema, retarded growth, ptosis, webbing of the neck, a low posterior hairline, cubitus valgus and hypoplastic nipples. Turner (1938) described similar congenital malformations together with sexual infantilism but not lymphedema in seven females aged 15 to 23 years. Later work by Ullrich (1949) claimed to have found the pathogenetic basis of all cases with Ullrich-Turner syndrome in the myelencephalic bleb mutation of mice investigated by Bonnevie (1934).

Flavell (1943) first used the term 'Turner's syndrome in the male' to describe patients with hypogonadism in association with shortness of stature, webbed neck and abnormalities of the skeletal and cardiovascular systems. In 1963, Noonan recognised that this syndrome could occur in males and females and that it was nosologically and etiologically different from the Ullrich-Turner syndrome in females with the 45,XO karyotype. Ferguson-Smith (1965) has suggested that Turner's stigmata in males is caused by deletion of certain loci on the Y chromosome.

In males the penis and scrotum are generally of normal size or enlarged whilst testicular size is invariably decreased and cryptorchidism and anorchia have been reported (Heller, 1965). The seminiferous tubules are often small with moderate sclerosis, sometimes lined with only Sertoli cells ('testicular germinal dysgenesis' Fraccaro et al., 1961). Leydig cells have been reported to be absent, reduced or normal. Thus there may be a nosological problem dependent on the extent of dysgenesis within the testes.

The urinary excretion of gonadotrophin is usually elevated in such cases whilst the urinary excretion of 17-oxosteroids is low with no increase following HCG administration (Schoen, 1965).

Anorchia

These patients have a normal male karyotype (46,XY), normal prepubertal development of external genitalia, but lack development of secondary sex characteristics. The eunuchoidal stature characteristic of the older patients with anorchia may be apparent even in the prepubertal period. By definition these patiemts have no functioning testicular tissue although it is generally assumed that foetal testes must have been present and functioning throughout early masculine sex differentiation since absence of testes then would have resulted in a female phenotype (Jost, 1947). In some cases interstitial cells present in the retroperitoneal tissue or along the epididymis and Wolffian duct structures may partially compensate for anorchia (McDonald and Calams, 1958). In the majority the etiology is obscure.

During adolescence the urinary gonadotrophin excretion is elevated in all patients with anorchia. The urinary 17-oxosteroid excretion is decreased and the administration of HCG has no effect on either the 17-oxosteroid excretion or on furthering masculinization (Bergada et al., 1962). Recently Rivarola et al. (1970) have measured plasma testosterone in five boys with anorchia before and after the administration of HCG. They found basal levels of plasma testosterone (37 ± 23 ng per 100 ml) that did not rise on HCG administration.

Cryptorchidism

Cryptorchidism is one of the most common congenital defects in the young male. Undescended testes may be classified as totally undescended, incompletely undescended or ectopic (ie descended along the wrong line). Cryptorchidism may be unilateral or bilateral and should be carefully distinguished from the highly retractile testis.

Scorer (1964) examined 3,612 male infants and found the incidence of undescended testes to be 21 per cent in premature infants, 2.7 per cent in full-term infants and 0.77 per cent after one year. Ward and Hunter (1960) found an incidence of undescent of 1.6 per cent in 5-year-old boys, whilst in the adolescent age group, 14 to 17 years, only 0.3 per cent had undescended testes. This closely approximates to the incidence found in 12,535,824 military recruits (0.28 per cent; Campbell, 1959).

Histologically the cryptorchid testis shows progressive deterioration. Incomplete development of the seminiferous tubules and failure of the normal maturation of the germinal epithelium occur. Sertoli cells and Leydig cells are usually normal (Mancini et al., 1965) (Figure 1.3). If the testis remains undescended after puberty, interstitial fibrosis and tubular hyalinization occur and spermatogenesis is absent (Mancini et al., 1965).

Bilateral cryptorchidism associated with hypogonadism may be caused by prenatal hormone disturbances (Johnston, 1968) and genetic factors may be indicated by the familial occurrence of this disorder. Orchidopexy aims at preserving the testicular elements and hopefully the functional potential. Surgical intervention restores the testis to the normal scrotal position but the function of the testis can only be normal if no deterioration has occurred earlier.

Llaurado and Dominguez (1963) have shown decreased synthesis of testosterone from progesterone and subnormal production of testosterone in response to administered HCG in cryptorchid rats. Similar studies by Inano and Tamaoki (1968a) have shown a 60 per cent reduction in the activity of Δ^5-3β-hydroxysteroid dehydrogenase. These results are in agreement with the decreased testosterone secretion (Eik-Nes, 1966; Amatayakul et al., 1971) and decreased spermatogenesis (Clegg, 1965; Amatayakul et al., 1971) noted in experimental cryptorchid rats, and dogs rendered cryptorchid. Recently Hamilton et al. (1970) have

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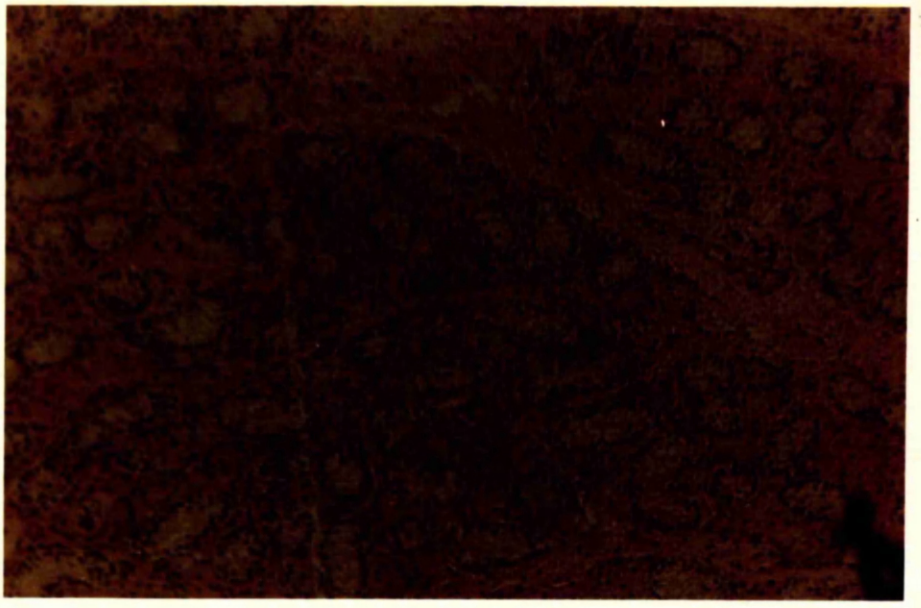

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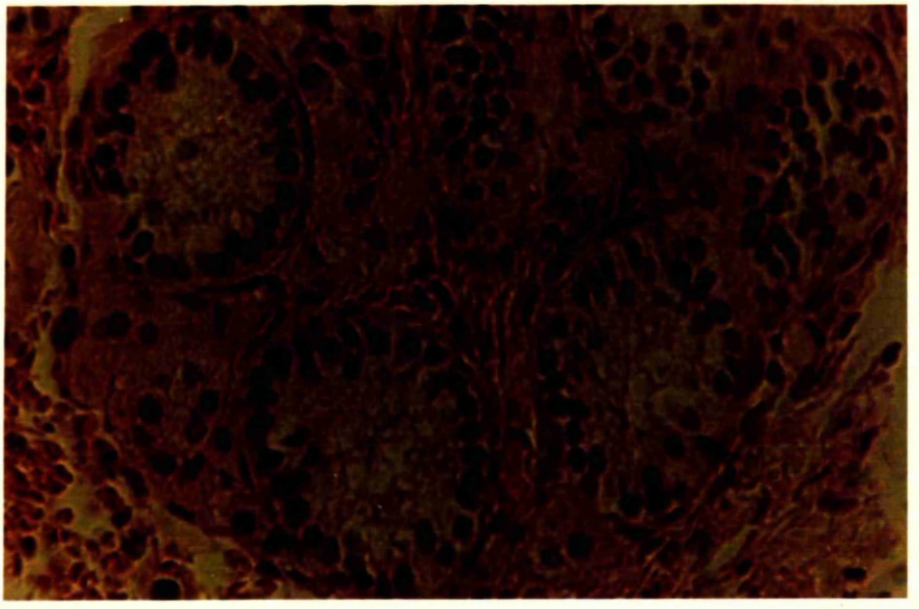

Figure 1.3 Testicular section from a patient with bilateral undescended testes (Protocol 53, Chapter 5) aged 11 years 6 months at biopsy. Note extremely reduced diameter of the seminiferous tubules (A) when compared with 'near-normal' testis (Figure 1.5), and normal appearance of Leydig cells and Sertoli cells (B).

reported a 3β-hydroxysteroid dehydrogenase deficiency in cryptorchid human testes.

Recently Rivarola et al. (1970) have reported normal Leydig cell function from determination of plasma testosterone before and after administration of HCG (800-5,000 I.U. per day for five days) in prepubertal patients with unilateral and bilateral cryptorchidism.

Germinal Cell Aplasia

Del Castillo et al. (1947) described the syndrome characterized by absence of the germinal epithelium without impairment of the Sertoli or Leydig cells. Phenotypically these patients were of normal stature, had small testes of normal consistency, normal genital development and were without gynaecomastia. Histologically the testes showed a complete absence of the germinal epithelium, the seminiferous tubules were of smaller diameter and contained only Sertoli cells. The Leydig cells appeared normal and there was no hyalinization of the basement membrane. Gonadotrophins were originally described as normal (del Castillo et al., 1947) although more recent reports indicate that the urinary excretion of these hormones is increased.

Patients with germinal aplasia have normal negative sex chromatin patterns in association with a normal male karyotype. This suggests that the syndrome is not genetically determined. Del Castillo et al. (1947) have suggested a failure of migration of the primordial germ cells into the developing testis during foetal development.

Seminiferous Tubule Failure

The majority of males with infertility have normal secondary sexual characteristics and no evidence of endocrine disease. Whilst some may have mechanical blockages caused by congenital defects of the epididymis and vas deferens (Girgis et al., 1969), the remainder have abnormalities of the seminiferous tubules. Although in the majority of such cases with

seminiferous tubule failure there is no known aetiology, failure in some cases is secondary to specific diseases.

Mumps orchitis occurring in the adolescent or adult may result in subsequent testicular atrophy (Scott, 1960). Testicular biopsy shows atrophy of the seminiferous tubules whilst the Leydig cells normally remain unimpaired, except in extreme cases where complete loss of testicular function may occur. Serum FSH concentration is elevated (Rosen and Weintraub, 1971) this in proportion to the tubular atrophy.

Irradiation damage to the germinal epithelium is well documented in the literature. The spermatogonia are much more sensitive to radiation than the Sertoli or Leydig cells (Mandl, 1964) although recent work has shown a biochemical lesion in the androgen biosynthetic pathways together with an increased gonadotrophin secretion and decreased testicular sensitivity to gonadotrophin (Ellis, 1970).

Spermatic failure may also be secondary to other factors including paraplegia and varicoceles of the spermatic cord (Hall et al., 1969).

PITUITARY GONADOTROPHIN FAILURE

Hypogonadism and delayed puberty secondary to a failure of pituitary gonadotrophin secretion can be divided into two types: congenital lack of gonadotrophins alone and those associated with general pituitary disease or lesions (Table 1.II). Isolated gonadotrophin failure can be further subdivided into hypogonadotrophic hypogonadism, the fertile eunoch syndrome, and gonadotrophin failure associated with various congenital disorders.

Hypogonadotrophic Hypogonadism

The aetiology of hypogonadotrophic hypogonadism is unknown. These patients are characterized by hypogonadism, delayed puberty and eunuchoidism. An association with anosmia, hyposmia, midline facial and neurological abnormalities is common. These associated defects suggest central nervous system abnormalities which may prevent the gonadotrophin-releasing mechanism

from functioning normally, so disrupting the normal pubertal processes. In these cases the seminiferous tubules are immature and spermatogenesis is generally absent. Sertoli cells may be present (Bardin et al., 1969) or absent (Paulsen, 1968) and the Leydig cells are poorly differentiated.

Total gonadotrophin excretion is low or undetectable (Heller and Nelson, 1948; Bardin et al., 1969) and plasma gonadotrophins fail to respond to clomiphene (Bardin et al., 1969). Generally Leydig cell function is reduced or absent. The plasma concentration of testosterone is very low and the response to administered HCG variable, although generally poor (Bardin et al., 1969).

Fertile Eunuch Syndrome

The term 'fertile eunuch' introduced by McCullagh et al. (1953) is perhaps a misnomer, since only two authenticated cases of fertility have been reported. The characteristics of this syndrome are eunuchoidism with normal-sized testes, spermatogenesis in the absence of Leydig cells and an androgenic response to administered HCG.

McCullagh et al. (1953) postulated an isolated defect of LH secretion with normal FSH secretion. Later work by Faiman et al. (1968) confirmed this theory. They found normal urinary and plasma levels of FSH with undetectable LH before and after clomiphene.

Pituitary Gonadotrophin Failure Associated with Congenital Disorders

Secondary hypogonadism is frequently associated with other congenital defects in the Laurence-Moon-Biedl syndrome and the Prader-Willi syndrome.

The Laurence-Moon-Biedl syndrome is transmitted as an autosomal recessive characteristic and is characterized by hypogonadism, obesity, mental retardation, polydactylism and retinitis pigmentosa. In males the testes show histologically immature seminiferous tubules and poorly differentiated Leydig cells (Franke, 1950) (Figure 1.4). The gonadal dysfunction may be due to either primary testicular failure or hypogonadotrop hypogonadism (Reinfrank and Nichols, 1964).

Figure 1.4 **Testicular section from a patient with the Laurence-Moon-Biedl syndrome (Protocol 59, Chapter 5) aged 5 years 5 months at biopsy. Although this testis shows the characteristics of the syndrome, immature seminiferous tubules and sparse Leydig cells, these characteristics are found in the testes of normal boys of this age.**

The Prader-Willi syndrome is characterized by hypogonadism, hypomentia, hypotonia, obesity and pleasant facial features. Diabetes mellitus occurs in approximately half the patients post-pubertally (Dunn, 1968). Males also have a hypoplastic scrotum, undescended testes and delayed puberty. The urinary excretion of 17-oxogenic steroids is elevated and this is consistent with the obesity noted in these cases whilst the urinary excretion of 17-oxosteroids is very low and consistent with reduced testicular Leydig cell function (Dunn, 1968; Monnens and Kenis, 1965).

Anterior Pituitary Disease

Patients with panhypopituitarism usually show only immaturity and small stature in childhood. Puberty does not occur at the expected time and the genitalia remain infantile. Histologically the testes are similar to other types of prepubertal gonadotrophin failure and show immature seminiferous tubules, poor development of the germinal epithelium and poorly differentiated Leydig cells (Albert et al., 1953). Urinary gonadotrophin excretion cannot be detected and the excretion of urinary 17-oxosteroids is low. Plasma testosterone is very low and the response to administered HCG is subnormal (Stuiver et al., 1966).

TESTICULAR FUNCTION IN PHENOTYPIC FEMALES

Tests of Leydig cell function and reserve, although originally designed to study hypogonadism, are applicable to several types of phenotypic females including those with the testicular feminization syndrome, XO/XY mosaicism and true hermaphroditism. Such patients present varying degrees of masculinization and the gonads may range from normal testes to undifferentiated streaks.

TESTICULAR FEMINIZATION SYNDROME

The complete form of testicular feminization is characterized by a female phenotype with normal feminization, near normal breast development,

female external genitalia (small or normal clitoris, blind ending vagina), absent Müllerian derivatives and sparse or absent pubic and axillary hair in association with a 46,XY karyotype and cryptorchid testes (Morris, 1953; Morris and Mahesh, 1963). Taillard and Prader (1957) have reported similar patients who show some degree of secondary sexual development and partial virilization, especially at puberty, and they classify these as incomplete forms of the syndrome.

Complete Testicular Feminization Syndrome

These patients fail to virilize at adolescence even though the testes are able to synthesize testosterone normally (Griffiths et al., 1963; Sharma et al., 1965) and maintain plasma levels of testosterone similar to those of normal males (French et al., 1965; Southren et al., 1965). French et al. (1965, 1966) have postulated an antenatal target organ defect in these patients. Such a target organ defect in foetal development would explain the failure of penile urethral formation, the failure of androgens to inhibit the formation of breast anlage (Goldman and Neumann, 1969) and the presence of feminine psychosexual differentiation of the brain (Money et al., 1968).

Histologically the gonads have been described extensively and interpreted as resembling immature (Pion et al., 1965), foetal (Turner, 1964), infantile (Neubecker and Theiss, 1962), or undescended testes (Morris, 1953). The seminiferous tubules are usually tightly packed, often devoid of a tubular lumen without evidence of spermatogenesis, peritubular fibrosis, or hyalinization. The Leydig cells are generally believed to resemble those of the mature foetus and do not contain Reinke's crystals. Leydig cell hyperplasia is often described as secondary to the elevated gonadotrophin in the absence of a negative feedback control (Morris, 1953).

Gual et al. (1962) studied 'abnormal' testicular tissue from a patient with the testicular feminization syndrome and reported that

Δ^5-androstene-3β,17β-diol was not further metabolised to testosterone. Griffiths et al. (1963), incubating tissue from similar patients, reported normal testosterone synthesis from Δ^4-steroid precursors although they were unable to identify oestrogens in the incubation effluent of their experiments. Normal testosterone biosynthesis in testicular tissue from a patient with the testicular feminization syndrome was also found by Morris and Mahesh (1963) whilst Sharma et al. (1965) confirmed normal testosterone biosynthesis in such patients and demonstrated oestrogen biosynthesis from androgen precursors. Further work by French et al. (1965, 1967), Kase and Morris (1965), David et al. (1965), and Gwinup et al. (1966) has confirmed the normal testosterone biosynthesis in the testicular feminization syndrome.

Patients with testicular feminization have a normal or slightly elevated excretion of 17-oxosteroids. The secretion of gonadotrophins may be either normal or elevated and increases following castration (Morris, 1953; Morris and Mahesh, 1963). The urinary excretion of testosterone (Rivarola et al., 1967; Jeffcoate et al., 1968), and the fractionated 17-oxosteroids (Morris and Mahesh, 1963; Philip and Sele, 1965), are within the normal male range. Similarly the plasma concentration of testosterone, dehydroepiandrosterone sulphate and androsterone sulphate are in the normal male range. They increase on administration of HCG but do not decrease on administration of dexamethasone. This is in accord with their testicular origin (Pion et al., 1965; Morris and Mahesh, 1963).

Urinary oestrogens are generally described as being in the low female range or male range and there is no evidence of a cyclic activity of oestrogen excretion (David et al., 1965).

Partial Testicular Feminization Syndrome

These patients often fail to feminize at puberty and may even virilize further. The external genitalia are often ambiguous and there is an associated hypospadias. The size of the phallus is variable, often adequate for

functioning as a male organ, or described as an enlarged clitoris. Of the published cases, approximately half have been raised as males and half as females, whilst four changes of rearing sex have been effected (Park and Jones, 1970).

The aetiology of this syndrome is not known, although both testicular deficiency and partial non-responsiveness of the target organ have been suggested as likely causes.

Histologically the testes of the prepubertal patient are normal although slight hyalinization of the tubules may occur. In the pubertal and adolescent age groups the testes show signs of atrophy as seen in undescended testes (hyalinization of the seminiferous tubules, decrease in Sertoli cell numbers, Leydig cell hyperplasia and absence of spermatogenesis) (Figure 1.5).

Bowen et al. (1965) reported a normal urinary excretion of 17-oxosteroids but a decreased excretion of testosterone glucuronoside in such patients. Urinary gonadotrophins have been described as normal or increased. Recently Rosenfield et al. (1971) found plasma testosterone and plasma androgens in the male range with an increase on administration of HCG. They concluded that their patient showed partial target organ insensitivity to testosterone, whilst Saez et al. (1971) postulated a defect in testicular 17β-reductase from studies of plasma and urinary androgen metabolites in similar patients.

XO/XY MOSAICISM

XO/XY mosaicism produces a spectrum of phenotypes ranging from Turner's syndrome without evidence of masculinization (XO effect) to a normal male phenotype (XY effect). These extreme phenotypes will not be considered here but rather the phenotypes of intersex, presumably due to commensurate effects of both the XO and XY cell lines.

Sohval (1964) introduced the term 'mixed' gonadal dysgenesis to classify cases with a unilateral testis and a streak gonad on the

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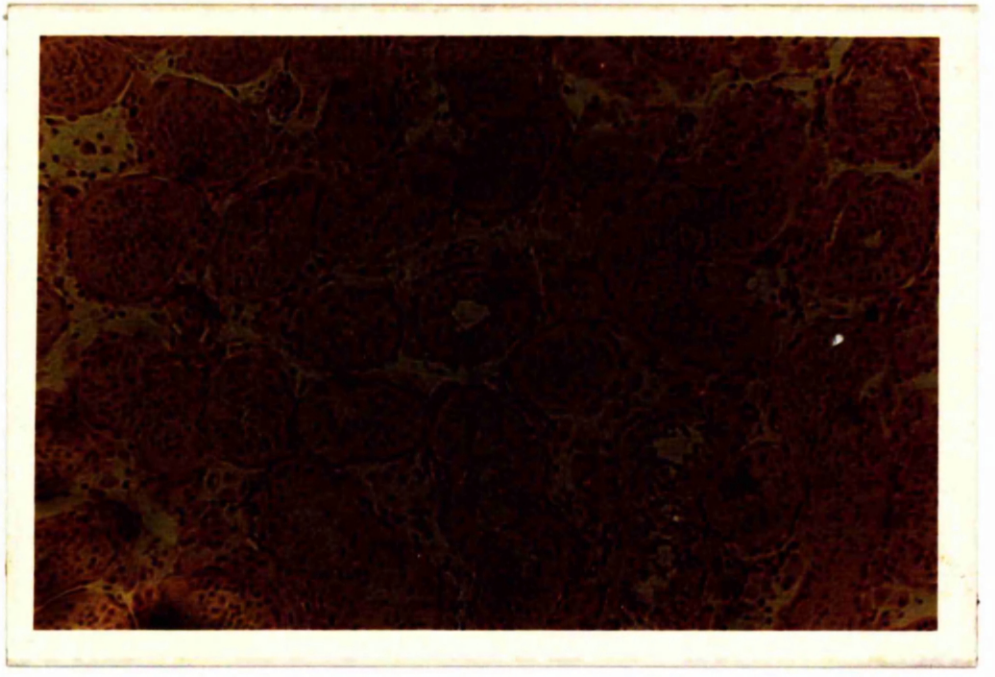

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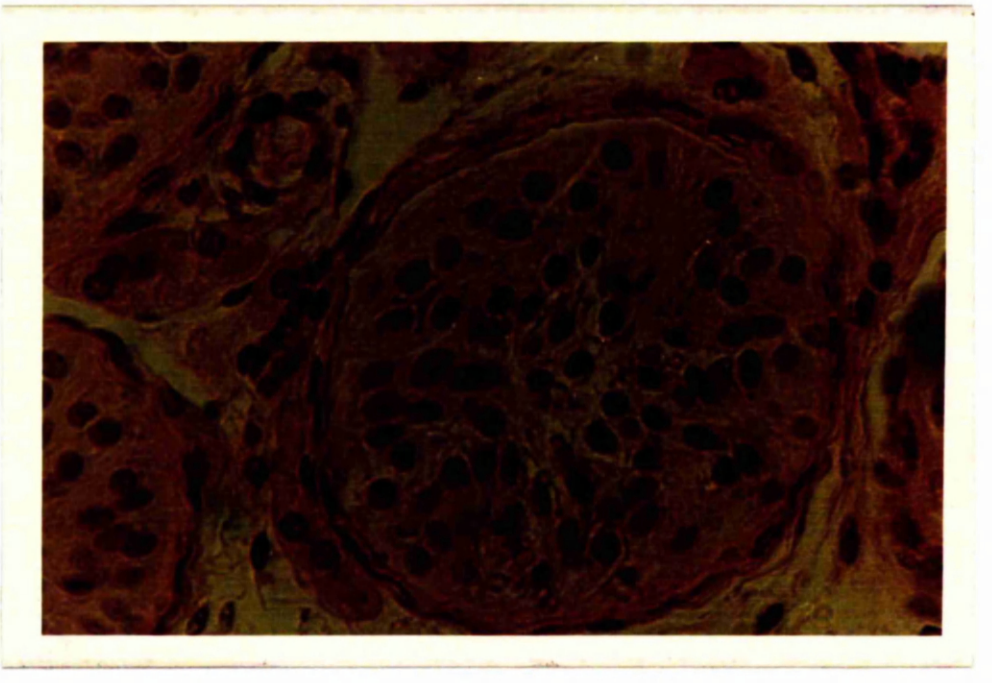

Figure 1.5 Testicular section from a patient with female phenotype and partial form of the testicular feminization syndrome. The patient was aged 12 years 6 months at biopsy. Note the abundance of Leydig cells between the normal-sized seminiferous tubules (A), the early hyalinization of the seminiferous tubular membrane and hyperplastic Leydig cells (B) characteristic of this syndrome.

contralateral side. Such patients may present as phenotypic males or females. The female phenotype is characterized by poorly developed secondary sexual characteristics, with evidence of virilization (enlarged clitoris, hypertrichosis and masculine voice) whilst the male phenotype is characterized by hypospadias, bifid scrotum, small penis and normal masculine body form. Both male and female phenotypes have negative sex chromatin, a unilateral testis with a contralateral streak gonad and unilateral or bilateral Müllerian duct derivatives (fallopian tube, uterus) and often stigmata of Turner's syndrome.

The competence of the unilateral testis to suppress the development of the Müllerian ducts is of interest in several cases where a unicornate uterus and single fallopian tube have been described (Zourlas and Jones, 1965; Hortling et al., 1970). In cases where bilateral Müllerian derivatives have been described (Sohval, 1964; Mellman et al., 1963) a deficiency of Müllerian duct inhibiting substance is implied in addition to a failure of the foetal testis to produce androgen (ambiguous genitalia).

Histologically the testis is variable and has been described as normal (Bergada et al., 1962), intermediate between 'pure' gonadal dysgenesis and 'true' hermaphroditism (Sohval, 1964), dysgenic (Mellman et al., 1963) or Sertoli cells only (Hortling et al., 1970). Spermatogenesis is normally absent (Sohval, 1964; Hortling et al., 1970) and the tubules may be hyalinized (Figure 1.6) or normal (Hortling et al., 1970). Leydig cells are often hyperplastic and clumped (Figure 1.6).

The streak gonad is composed predominantly of fibrous connective tissue often resembling ovarian stroma. Tubular structures representing remnants of rete or mesonephric ducts are infrequently present (Willemse et al., 1962). Leydig-type cells have been noted (Hortling et al., 1970). The streak gonad represents the rudimentary remnant of an embryonic gonadal ridge before testicular or ovarian differentiation.

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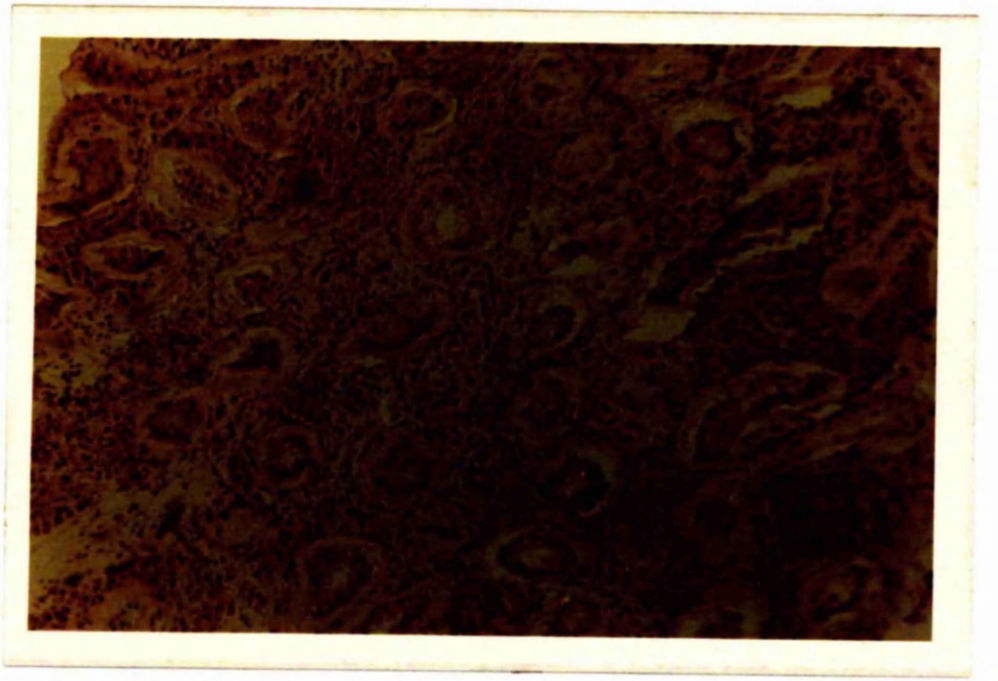

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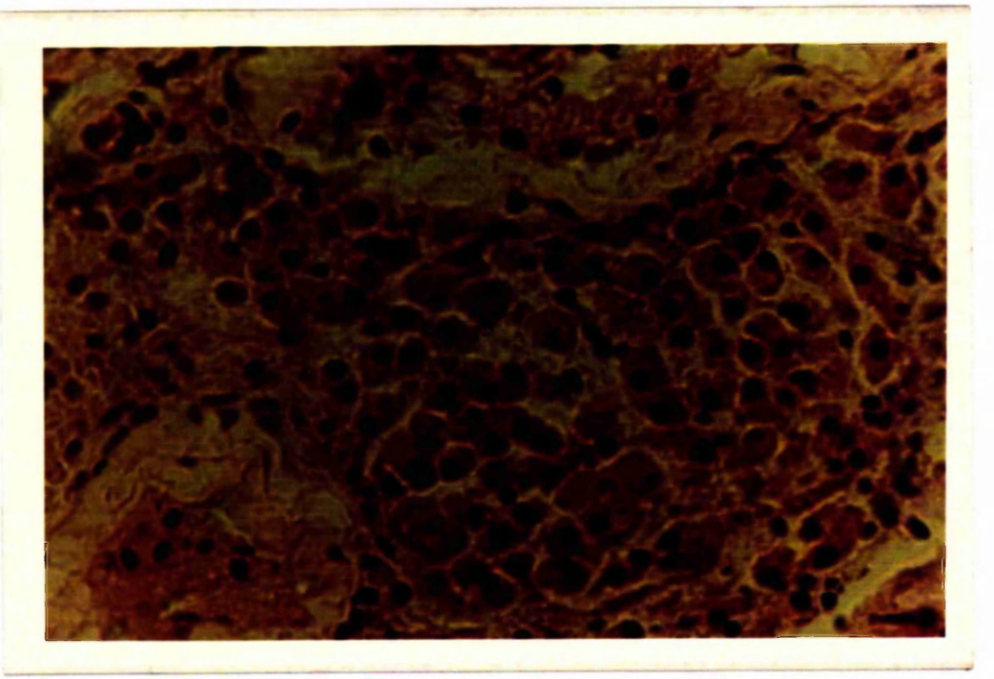

Figure 1.6 Testicular section from a patient with the XO/XY syndrome and female phenotype (Protocol 6, Chapter 5) aged 12 years 3 months at biopsy. Note the severe hyalinization of the seminiferous tubules and abundant hyperplastic Leydig cells (A) often clumped (B).

TRUE HERMAPHRODITISM

By definition patients with true hermaphroditism contain both ovarian and testicular tissue. Such patients are generally classified according to the position of the gonadal tissue. The lateral variety (testicular tissue one side, ovarian tissue contralaterally), the bilateral variety (testicular and ovarian tissue on both sides) and the unilateral variety (testicular and ovarian tissue on one side, testicular or ovarian tissue contralaterally) are noted.[1] Ovotestes are found in some such patients, particularly in the bilateral variety (either unilaterally or bilaterally) and in the unilateral variety of hermaphroditism.

These patients are characterized by a complete range of developmental abnormalities of both the internal and external genitalia and cannot be distinguished clinically from other forms of intersexuality. Somatic abnormalities are occasionally present in hermaphroditism with chromosomal mosaicism. The degree of differentiation of either the Müllerian or Wolffian ducts and the extent of masculinization of the external genitalia are an index of foetal testicular function.

Chromosomal analysis has shown that the majority of such cases have an XX karyotype although other karyotypes have been described (XY, XX/XY, XO/XY, XX/XXYY and other forms of mosaicism). Josso et al. (1965) investigated a patient with XX/XY mosaicism and concluded that the mosaicism was related to double fertilization of the ovum. In cases with either XX or XY karyotypes it is possible that mosaicism has been overlooked although further investigations in such patients have failed to find a second cell line (Root et al., 1964).

Histologically the separate ovary or testis in these patients have been described as normal although Overzier (1963), in a review of 171 patients, reported that patients with negative sex chromatin had generally underdeveloped gonads. The ovotestis is of interest since it usually appears in

the prepubertal patient as a foetal or immature testis separated by fibrous tissue (tunica albuginea) from typical ovarian stroma rich in oocytes and primordial follicles (Figure 1.7). After puberty the germ cells degenerate and tubular atrophy with hyalinization is often seen. Leydig cell hyperplasia is noted post-pubertally and is probably secondary to the almost constantly elevated serum gonadotrophin.

THE NATURE OF THE PROBLEM

What is to be the treatment of these patients with ambiguous sexual development? Does one sex predominate and if so how do we recognize it? Whatever the sex of rearing, the degree of virilization of the external genitalia at puberty may necessitate further surgery or hormone treatment unless such a prognosis is available for the neonate.

In the prepubertal patient with hypogonadism the problem is to make the external genitalia conform to the sex of rearing. Again surgery or hormone treatment are usually indicated although in some cases this may not have the desired effects. Ideally we need to know the functional integrity of the gonads, the levels of hormone production and their metabolism within the target organ tissues so that a prognosis can be given for subsequent development of the secondary sexual characteristics at puberty.

THE PRESENT INVESTIGATION

In this investigation various parameters of Leydig cell function and reserve were determined by response to administered human chorionic gonadotrophin (HCG). The peripheral plasma concentration of testosterone and the daily urinary excretion of total 17-oxosteroids, testosterone and individual androgen metabolites were quantitated, before and during administration of HCG, in a variety of pathological conditions. Results are presented of an investigation of 80 patients and later (Chapter 4) I shall attempt to enunciate the principles on which I think treatment may be justified.

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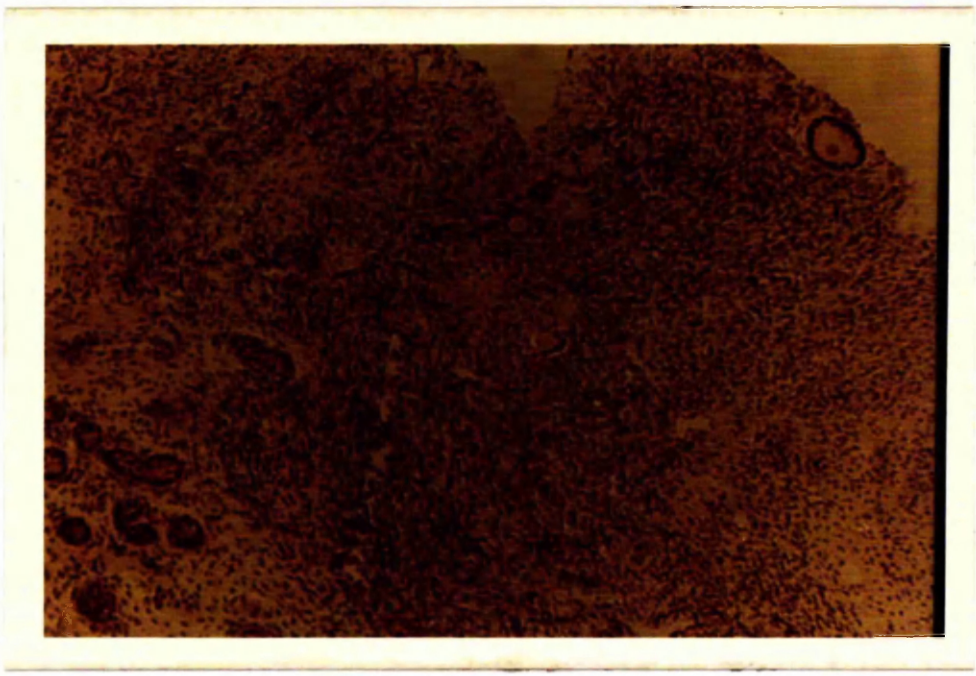

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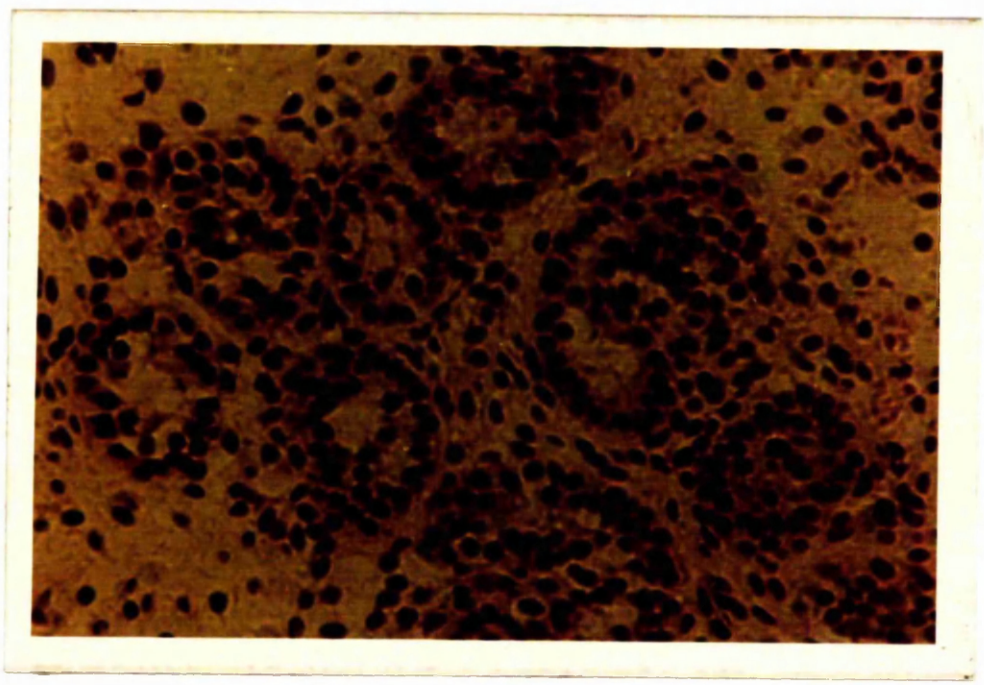


Figure 1.7 Section of an ovotestis from a patient with the XX/XY genotype and true hermaphroditism (Protocol 5, Chapter 5) aged 6 years at biopsy. Note the combination of typical ovarian stroma (with follicle) with seminiferous tubules within this tissue (A). The testicular component is poorly developed (B), the seminiferous tubules resembling those seen in the foetus.

1

CHAPTER 2

METHODOLOGY

MATERIALS

RADIOACTIVE-LABELLED STEROIDS

All radioactively labelled steroids used in this investigation, with the exception of [1,2-^{3}H]androsterone, were obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England. [1,2,6,7-^{3}H]Testosterone, specific activity (S.A.) 87 Ci/mM, was used in tracer amounts to estimate the recovery of methods developed to quantitate testosterone in plasma and urine. [4-^{14}C]Testosterone (S.A. 59 mCi/mM) was used to test the stability of the tritium labelled testosterone, whilst [7α-^{3}H]dehydroepiandrosterone sulphate (S.A. 4.6 Ci/mM) was used to test the hydrolysis technique. [1,2-^{3}H]Androsterone (S.A. 40 Ci/mM) was obtained from New England Nuclear, Albany Street, Boston, Mass., USA, and used in tracer amounts to estimate the efficiency of derivative formation of androsterone-3-heptafluorobutyrate, the internal standard used for gas-liquid chromatography quantitation of testosterone in biological samples.

All radioactively labelled steroids required purification by thin layer chromatographic (TLC) systems before use. A single peak of Gaussian form obtained on radiochromatogram scanning was taken as the indication of purity. Generally development in at least three separate TLC systems was required to obtain such a peak. Then as much as 5 per cent of the radioactivity in the commercially supplied radioactively labelled steroid was removed as impurity. All purified radioactively labelled steroids exhibited the same chromatographic mobility as a corresponding unlabelled reference standard. Working solutions of the purified radioactively labelled steroids were made with benzene:chloroform (6:1) for free steroids, and with ethanol for conjugated steroids. The working solutions were stored at 5°C.

UNLABELLED REFERENCE STEROIDS

Standard steroids were obtained from Koch-Light Laboratories Ltd (Colnbrook, Buckinghamshire, England) and Sigma London Chemical Co. Ltd

(Norbiton Station Yard, Kingston upon Thames, Surrey, England). Small quantities of epitestosterone (17α-hydroxyandrost-4ene-3one), Δ^4-androstene-3β,17β-diol and the glucuronoside and sulphate conjugates of epiandrosterone (3β-hydroxy-5α-androstane-17-one) were obtained from the M.R.C. Steroid Reference Collection (Chemistry Department, Westfield College, Hampstead, London NW3).

REAGENTS

Absolute ethanol (A.R. quality) was obtained from James Burrough Ltd (Fine Alcohols Division, Montford Place, London SE11, England). 'Peroxide-free' diethyl ether was purchased from May and Baker Ltd (Dagenham, Essex, England). Heptafluorobutyric anhydride and toluene 'scintillation grade' were obtained from Koch-Light Laboratories Ltd. PPO and POPOP, the primary and secondary solutes dissolved in toluene to produce the liquid scintillator, were obtained from Hopkin and Williams Ltd (Asschem, Redding Industrial Estate, Redding, Falkirk, Scotland). All other chemicals, unless otherwise noted in the text, were purchased from B.D.H. Chemicals Ltd (Poole, Dorset, England) and were normally of 'Analar' specification.

ENZYME PREPARATION

The enzyme β-glucuronidase, used for the hydrolysis of urinary steroid glucuronosides, was prepared in this laboratory according to the M.R.C. method (1963). Limpets (Patella vulgata) were collected from the rocky shore of the Clyde Estuary, two hours after high tide and immediately brought back to the laboratory in sea water. The molluscs were removed from their shells and the black visceral hump was dissected from the muscular foot. Batches of approximately 150 g of visceral humps were homogenized for two minutes with twice the volume of ice-cold water. After centrifugation (1000 r.p.m.) the supernatant was decanted and acetone added to bring the concentration to 60 per cent acetone in water in order to precipitate the proteinous enzyme. The mixture was allowed to stand overnight in the

refrigerator (5°C) at this stage. The supernatant was then removed after centrifugation at 1000 r.p.m. and discarded. The acetone precipitate was resuspended in acetone, washed by thorough mixing and centrifuged. The washing and centrifugation were repeated until all the chromogenic material had been removed by the acetone. The precipitate was dried at 25°C, powdered and assayed for β-glucuronidase activity.

Assay of β-Glucuronidase Activity

The method developed by Talalay et al.[1] (1946) was used to assay the potency of the β-glucuronidase extract. The limpet powder (200 mg) was dissolved in deionised water (40 ml) and 1 ml of the resulting suspension was made up to 12.5 ml with deionised water. 0.5 ml of this dilute solution (equivalent to 200 µg of enzyme powder) was used for estimation of β-glucuronidase activity.

0.1 M acetate buffer (4 ml) (5.785 g sodium acetate and 3.25 ml glacial acetic acid per litre of deionised water, adjusted to pH 4.6) was placed in four tubes. Phenophthalein glucuronoside (50 µg) was added to the two experimental tubes but not to the two control tubes. All tubes were then placed in a water bath at 38°C and allowed to equilibrate. The enzyme solution (0.5 ml) was added to each tube at timed intervals, mixed, stoppered and incubated for exactly 60 minutes. The reaction was stopped and the colour developed in all tubes by the addition of 0.4 M glycine buffer (5 ml) (16.3 g aminoacetic acid, 12.65 g sodium chloride and 10.9 ml concentrated sodium hydroxide 1:1 (w/v)). Finally, phenolphthalein glucuronoside (50 µg) was added to the two control tubes. The optical densities of the experimental and control tubes were read against standards of phenolphthalein (5-30 µg) in a Unicam SP600 spectrophotometer (Pye Unicam Ltd, York Street, Cambridge, England) set at 540 nm and employing matched glass cuvettes of 10 mm light path (Starna Ltd, Queens Road West, London E13, England). The potency of β-glucuronidase is then expressed in Fishman Units per gramme enzyme

preparation where one Fishman unit is equivalent to 1 µg phenolphthalein liberated from the substrate (phenolphthalein glucuronoside) by 1 g of enzyme powder.

Optical densities (OD) obtained on assay of the β-glucuronidase used in this investigation are given below. The spectrophotometer was zeroed against deionised water.

READINGS

OD 540 Control	003 ..... (A)
OD 540 25 µg Standard (phenolphthalein)	211 ..... (B)
OD 540 Experimental test	223 ..... (C)

CALCULATION

25 µg phenolphthalein ≡ (B - A) = OD 208

∴ 26.44 µg phenolphthalein ≡ (C - A) = OD 220

200 µg enzyme powder liberates 26.44 µg phenolphthalein

∴ 1 g enzyme powder liberates $\frac{26.44 \times 10^6}{200}$ µg phenolphthalein

ie 132,200 µg phenolphthalein

Thus 1 g enzyme powder contains 132,200 Fishman units.

BASIC METHODOLOGY

THIN LAYER CHROMATOGRAPHY

Merck pre-coated thin layer plates of silica gel 60 F_{254} (pre-coated layer thickness 0.25 mm) were purchased from Anderman and Co. Ltd (Central Avenue, East Molesey, Surrey, England). These plates were used without further washing or activation by heat for purification of testosterone extracts from plasma and urine.

Chromatography

Samples were applied to the thin layer using disposable micropipettes, a TLC tray and a spotting guide (Baird and Tatlock (London) Ltd, Asschem). After the application of samples, the chromatogram was developed in a TLC

tank lined with Whatman 3 MM chromatography paper (H. Reeve Angel & Co. Ltd, New Bridge Street, London EC4, England) using 100 ml of the appropriate solvent system for each development.

Detection of Steroids on TLC Plates by Ultraviolet Light

Δ^4-3-Oxosteroids were visualised on TLC plates using light emitted at 254 nm from a 'BTL Ultraviolet lamp for TLC' (Baird and Tatlock (London) Ltd). The fluor content of the silica gel, described above, facilitated detection. Other standard steroids were located after spraying with Rhodamine 6 G (0.1 per cent w/v in ethanol).

Radiochromatogram Scanning

Radioactively labelled steroids were located on TLC plates using a Panax 'Thin Layer Radiochromatogram Scanner, Model RTLS 1a' (Panax Equipment Ltd, Redhill, Surrey, England), in conjunction with a Smith's Flatbed Recorder (Smith's Industries Ltd, Industrial Instrument Division, Wembley Park, Middlesex, England). The detector gas was a mixture of argon (2 per cent) and propane (98 per cent) (British Oxygen Company Ltd, Special Gases Department, Deer Park Road, London SW19, England) regulated to a gas flow rate of 50 ml per minute. All plates were scanned at the 15 x 2 mm detector aperture, adjusted to a height of approximately 1 mm above the TLC plate. The detector voltage was set at 1,040 V, detector dead time 200 μS and discriminator bias at 10 mV. A time constant of 100 seconds and a scanning speed of 30 mm per hour were employed. The range of counting varied with the amount of radioactivity to be scanned.

Elution procedure

The silica on areas of thin layers to be eluted was loosened free from the TLC plate glass using a disposable scapel blade. The loosened silica was drawn by suction into a vacuum thimble filter. These filters consist of a sintered glass filter disc (approximately 1 cm diameter) encased in, and at one end of, glass tubing (4 cm long) to which at each

end narrower tubing (3 mm diameter, 2 cm long) is fused. These devices are a simplified version of the 'vacuum cleaners' described by Matthews et al. (1962) and are made to the above specification by Glass Appliances Ltd (488 Holburn Street, Aberdeen, Scotland). The eluent, normally methanol and/or ethyl acetate unless otherwise specified, was added to the inverted vacuum thimbles and the resulting eluate, which emerged through the filter, collected in test tubes (Figure 2.1).

LIQUID SCINTILLATION COUNTING

Radioactivity was measured by liquid scintillation counting using a Nuclear Chicago Mark 1 Scintillation Computer (Nuclear Chicago Corporation, Inc., Des Plaines, Illinois, USA) calibrated for simultaneous counting of ^{14}C and ^{3}H employing three channels. The scintillation fluid was toluene based and contained 4 g per litre PPO (2,5-diphenyloxazole) as the primary scintillator and 0.05 g per litre POPOP (1,4-di-(2-(5-phenyloxozolyl))-benzene as the secondary scintillator. Quenched standards (Nuclear Chicago Corporation, Inc.), containing an identical scintillation solution, were used to establish quench correction curves employing external standard channels ratio techniques and the external standard of ^{133}Ba incorporated into the scintillation counter.

All samples were counted in 20 ml scintillation fluid for 20 minutes. Aqueous samples containing free radioactively labelled steroids were blown to dryness under nitrogen at 40°C and then redissolved in the scintillation fluid, whilst radioactively labelled conjugated steroids were incorporated into the toluene based scintillator by dilution with 20 per cent methanol.

All results of quantitation by such liquid scintillation counting are expressed as disintegrations per minute (dpm) taking account of the channels ratio with the external standard.

GAS LIQUID CHROMATOGRAPHY

A Pye Series 104, Model 84 gas chromatographic system (Pye Unicam Ltd, York Street, Cambridge, England) was employed.

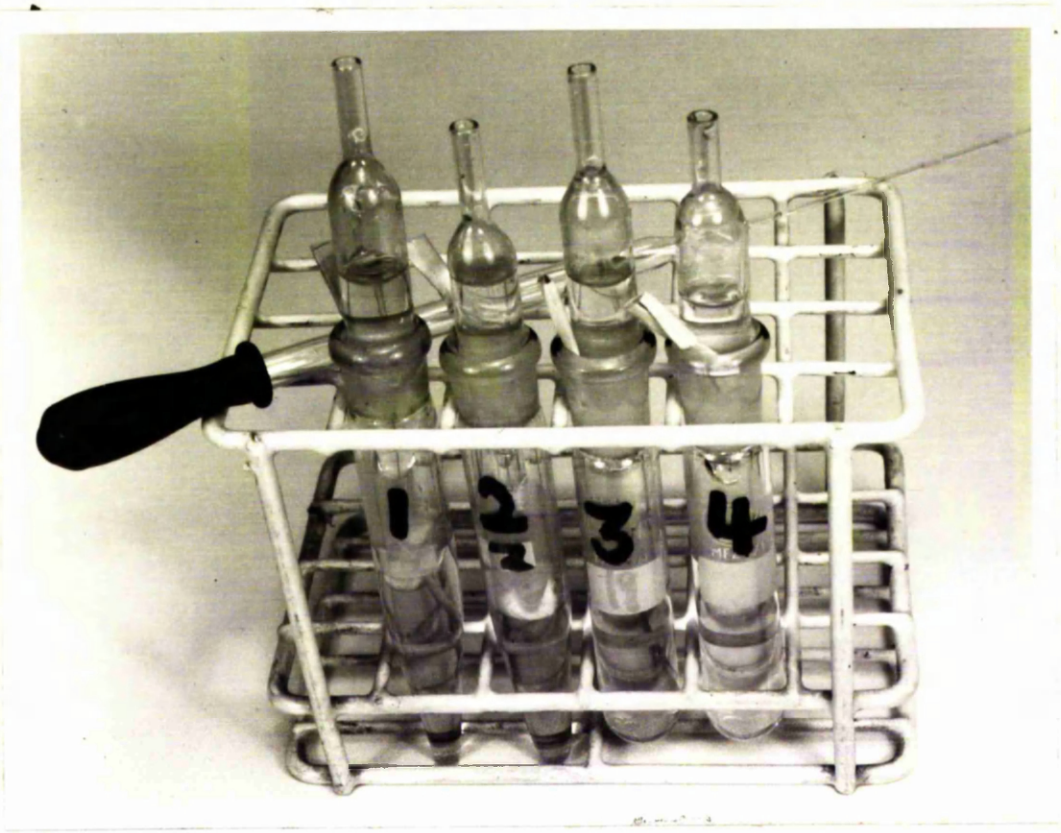


Figure 2.1 Extraction thimbles used for the collection and elution of silica from TLC plates. Elution of steroids from the silica was facilitated by percolation of solvent through the silica and sintered glass filter disc of the inverted thimble. The eluate was collected in test tubes.

Flame ionization detection (FID) was used to determine column separating powers and for detection of androgens and androgen metabolites in urine. The following gas flow rates were used with FID: oxygen-free nitrogen carrier gas - 40 ml per minute; hydrogen - 40 ml per minute; air - 650 ml per minute. Oxygen-free nitrogen and air were supplied by British Oxygen Co. Ltd, hydrogen was purchased from Messer Griesheim Ltd (43 Knights Hill, West Norwood, London SE27, England). Gas purification bottles were used with all gases.

Electron capture detection (ECD), using a ^{63}Ni source and a pulse voltage of 150 μS, was used in the final quantitation of extracts from urine and plasma. Column details and running conditions will be indicated in the text.

The samples were injected into the column using a 10 μl SGE syringe (Phase Separations Ltd, Queensferry, Flintshire, Wales). The recorder was a Philips Flat Bed Recorder, Model PM8000 (Pye Unicam Ltd) and a chart speed of 5 mm per minute (30 cm per hour) was used throughout the investigation

PRELIMINARY URINE TESTS

COLLECTION OF SAMPLES

Complete 24-hour urine collections were made from patients in hospital, under the supervision of the nursing staff. Specimens were collected in screw-top bottles to which chloroform (5 ml) was added as a preservative. Urine specimens were stored at $5^{o}C$.

DETERMINATION OF CREATININE

A modified Folin (1905) adaptation of the colour reaction of creatinine with alkaline sodium picrate first described by Jaffé (1886) was used for the determination of creatinine in 24-hour urine collections. Several of the modifications suggested by Bonsnes and Taussky (1945) were applied in the method.

After measuring the total urine volume, duplicate aliquots (0.5-2 ml) containing 0.7 to 1.2 mg creatinine were pipetted into 100 ml volumetric flasks. A reagent blank containing deionised water (1 ml) and a standard flask containing creatinine stock standard (creatinine 1 g/l in 0.1 N HCL) (1 ml = 1 mg) were prepared in similar flasks. Saturated picric acid (15 g picric acid per litre deionised water) (2 ml) was added to each flask followed by the addition, at timed intervals, of 1 M sodium hydroxide (1 ml). The flask contents were thoroughly mixed and allowed to stand exactly 10 minutes at room temperature before dilution to volume with deionised water. After dilution the contents were thoroughly mixed again and an aliquot transferred by Pasteur pipette to a cuvette of 10 mm light path. Optical densities were read at 490 nm on a Unicam SP600 spectrophotometer with zero set against the reagent blank.

The milligram equivalents of the test samples relative to the standard creatinine were calculated, and after adjustment for the aliquot of urine taken, and for the total 24-hour urine volume, the final results were expressed as mg creatinine per 24 hours.

Standard creatinine was found to obey the Beer-Lambert Law (Figure 2.2) at the concentrations used. This permitted direct quantitation of creatinine in test samples. The recovery of creatinine added to urine was consistently greater than 95 per cent. Results of typical recovery experiments are shown in Table 2.I. Duplicates were consistently within 3 per cent and inter-batch difference was less than 5 per cent.

CREATININE AS AN INDEX OF ACCURACY OF 24-HOUR URINE COLLECTIONS

Serial 24-hour urine specimens (4-5 days) were collected from children in hospital, under the supervision of the nursing staff. Considerable fluctuation was found in the volume of some 24-hour urine collections over the period of test. The completeness of certain specimens was therefore questioned.

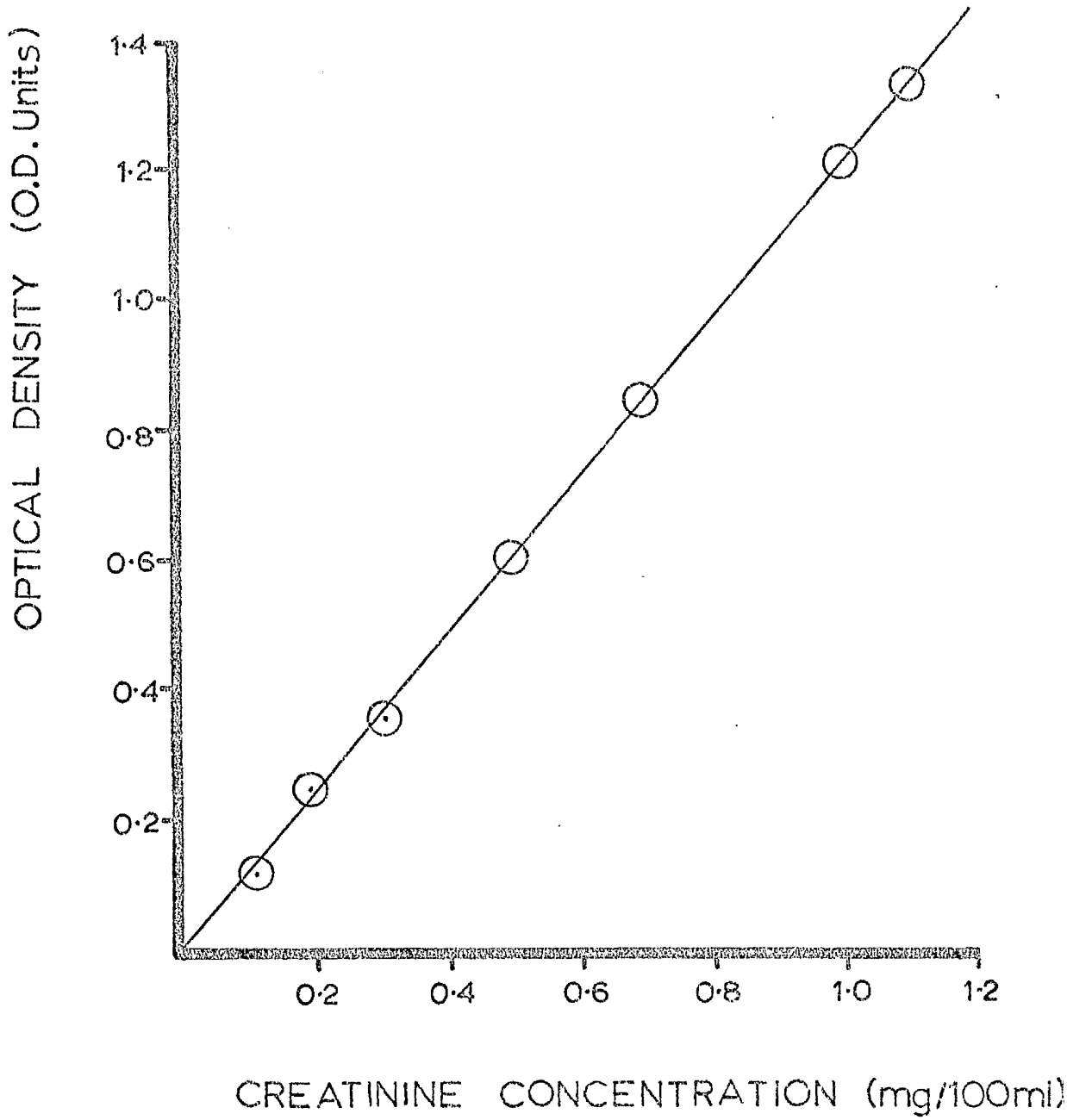


Figure 2.2 Optical density of creatinine as assayed by the Jaffé colour reaction. The results were corrected for reagent blank. Note the obedience of the results to the Beer-Lambert Law at creatinine concentrations between 0.5 and 1.1 mg/100 ml.

TABLE 2.I RECOVERY OF CREATININE ADDED TO URINE

Test	Optical Density @ 490 nm	Recovery of Creatinine
		%
Standard creatinine (1 mg)	1.20	-
Urine A	0.25	-
Urine A + creatinine (1 mg)	1.40	95.8
Urine A + creatinine (0.75 mg)	1.13	97.8
Urine A + creatinine (0.5 mg)	0.865	102.5
Urine A + creatinine (0.2 mg)	0.47	91.7
Urine A + creatinine (0.1 mg)	0.365	95.8
Urine A + creatinine (0.05 mg)	0.30	83.3
Urine A + creatinine (0.02 mg)	0.268	75.0

Recovery is acceptable (98.7 per cent) if aliquot analysed contains between 0.7 and 1.2 mg creatinine

As long ago as 1905 Folin (1905b) observed that although considerable individual variation was noticed, the daily quantity of urinary creatinine was remarkably constant for the individual and that this constancy of creatinine excretion could be used as a gauge of the completeness of a 24-hour urine specimen. Smith (1942) found the creatinine test of great use in assessing accuracy of collection of 24-hour specimens. Wray and Scott Russell (1960) and Paterson (1967) confirmed that the 24-hour excretion of creatinine was significantly more constant than the 24-hour urine volume.

In this work results of the determination of creatinine in serial 24-hour urine specimens were viewed critically. After rejection of significant outliers (Dixon, 1951) the mean creatinine excretion was calculated for each patient. Division of this mean creatinine excretion by the individual daily creatinine excretion gave the creatinine correction factor for each urine collection. All results of urinary steroid determinations were adjusted to a constant creatinine excretion over the test period by multiplication of the 24-hour urinary excretion of steroids by the creatinine correction factor. Approximately 2 per cent of the urine specimens were discarded as 'insufficient' for analysis.

GROUP DETERMINATION OF 17-OXOSTEROIDS

After measuring the total urine volume, duplicate 10 ml aliquots of undiluted urine were pipetted into boiling tubes of approximately 40 ml capacity. The tubes were placed, unstoppered, in vigorously boiling water and allowed to come to temperature. Concentrated hydrochloric acid (1 ml) was added to each tube, and boiling was continued for exactly 10 minutes to hydrolyse conjugated 17-oxosteroids.

The tubes were cooled rapidly in ice prior to extraction of steroids with chloroform (10 ml). After inversion of the tubes 20 times, the upper aqueous layer was removed using a Pasteur pipette attached to a water

suction pump. The chloroform was washed with 1 M sodium hydroxide (2 ml) and then with deionised water (2 ml). After each wash the aqueous layer was removed by suction. The organic phase was filtered through Whatman No. 1 phase separating paper to remove the water and an aliquot (5 ml) of the filtrate transferred to a 10 ml test tube. Evaporation was accomplished under nitrogen, the tubes standing in a water bath at 40°C. The 17-oxosteroid content of the dried residue was estimated colorimetrically by the Zimmermann reaction.

THE ZIMMERMANN REACTION

The James and de Jong (1961) modification of the Zimmermann reaction was used throughout this study. These authors recommend the use of tetramethylammonium hydroxide in preference to the less stable potassium hydroxide. A reaction mixture of 0.5 per cent dinitrobenzene and 25 per cent tetramethylammonium hydroxide in proportions 2:1 was used. The separate reagents were stored at 5°C until used.

The freshly constituted reaction mixture (0.25 ml) was added to the dried urine extracts from the above procedure. Duplicate reagent blank tubes and duplicate standard tubes containing dehydroepiandrosterone (10 µg) plus the reaction mixture were processed with each assay. The tubes were stoppered and incubated in the dark at 25°C for one hour (Figure 2.3). Ethanol (50 per cent) (2 ml) was pipetted into each tube and the Zimmermann colour extracted into diethyl ether (3 ml).

After separation of the phases, the upper ether layer was transferred by Pasteur pipette to a cuvette of 10 mm light path (Starna Ltd). Optical densities were read at 435, 515 and 595 nm (Figure 2.4) on a Unicam SP600 spectrophotometer with the zero reading set against diethyl ether. To correct for interfering chromatogens the Allen correction formula (1950) was applied to the optical densities at the wavelengths given above.

The corrected optical density at 515 nm (COD_{515}) is given by:-

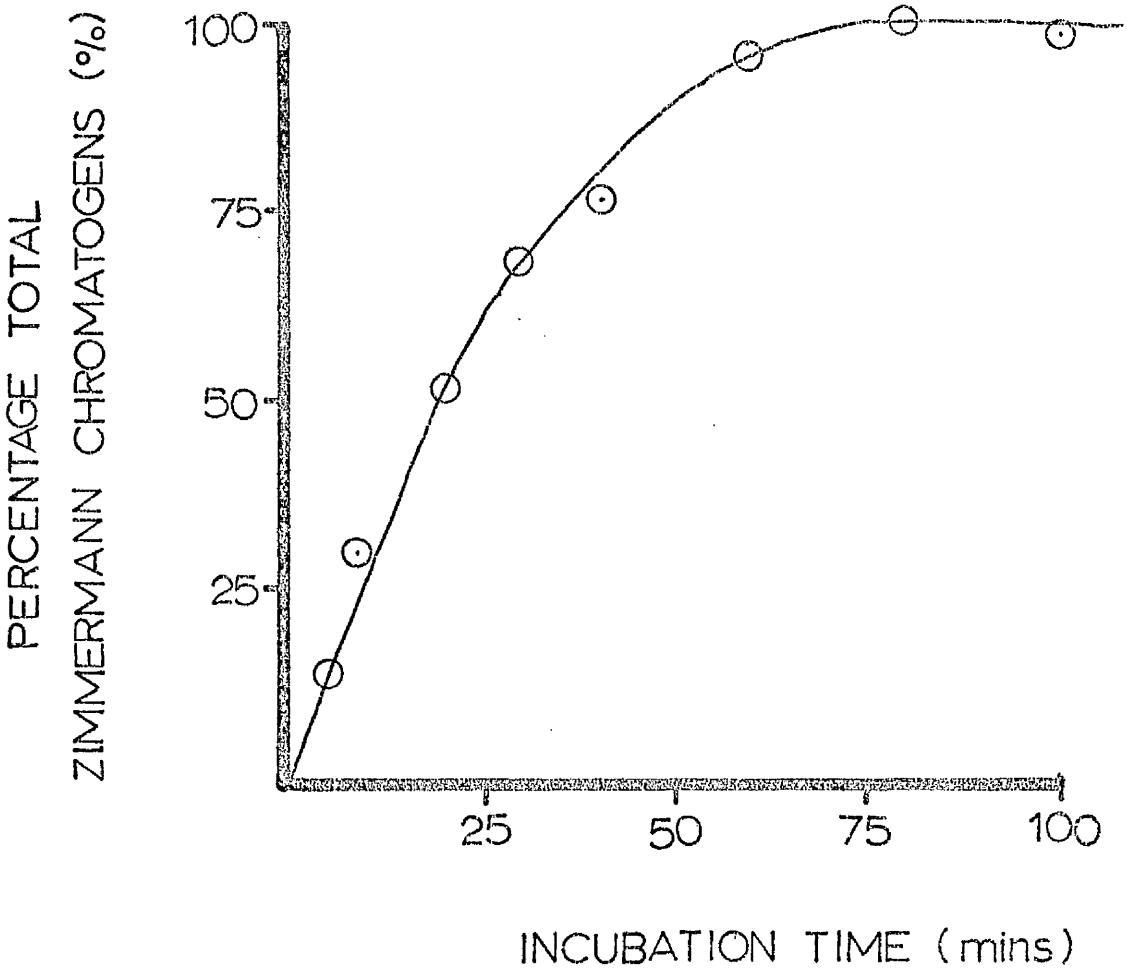


Figure 2.3 The increase in optical density of the Zimmermann chromatogens with time. Routinely the reactants were incubated for 1 hour at 25°C in the dark. The reaction mixture consisted of dinitrobenzene and tetramethylammonium hydroxide (2:1, v/v) (0.25 ml) added to the dry residue. Standard DHA (10 µg) was used in the above experiment and the Zimmermann chromatogens extracted into diethyl ether from the aqueous phase.

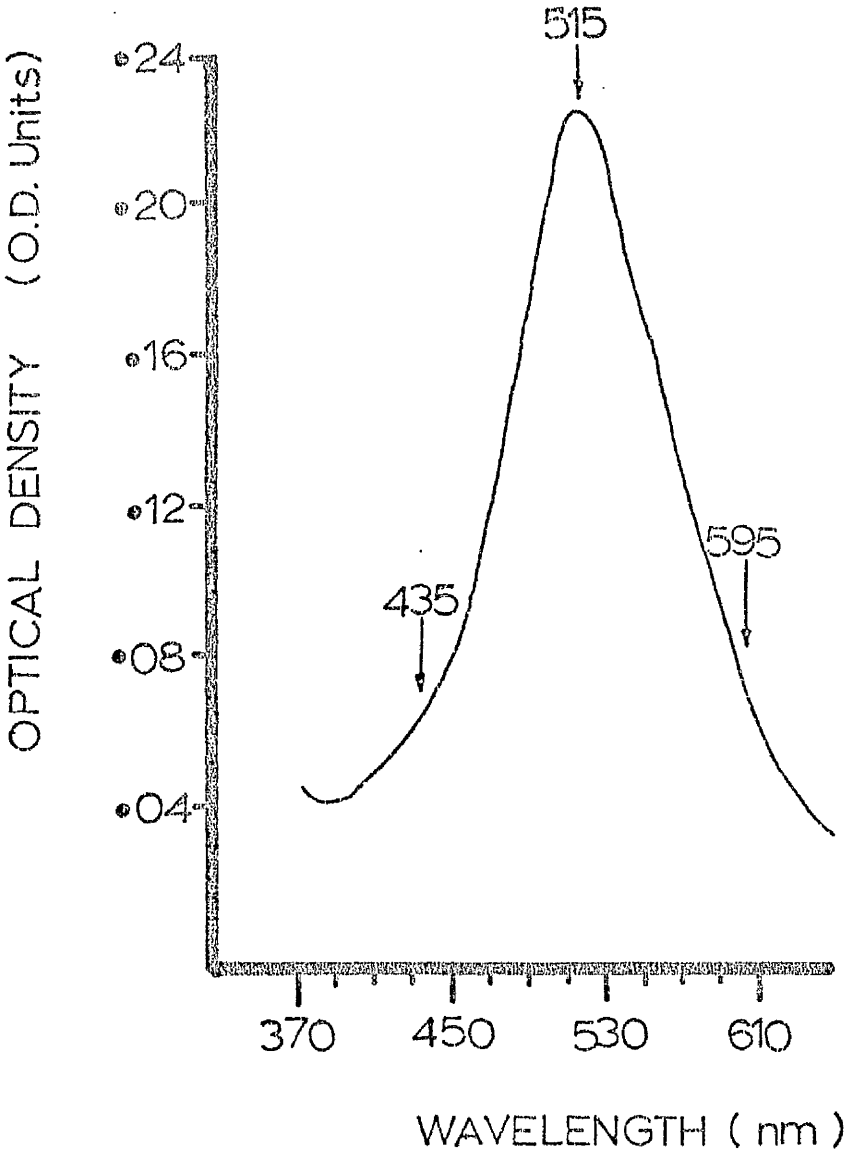


Figure 2.4 Optical density spectra of Zimmermann positive colour at varying wavelengths. DHA (15 µg) was incubated in the dark at 25°C for 1 hour with Zimmermann reagent (Figure 2.3). Routinely optical densities were measured at 435, 515 and 595 nm and the Allen correction (Allen, 1950) applied.

$$COD_{515} = OOD_{515} - \left[\frac{OOD_{435} + OOD_{595}}{2}\right]$$

Where OOD_{515} is the observed optical density at 515 nm

OOD_{435} is the observed optical density at 435 nm

OOD_{595} is the observed optical density at 595 nm

The reagent blank reading was subtracted from standard and test readings. The microgram equivalents of the test samples relative to the standard DHA were calculated, and after adjustment for the aliquot of chloroform extract taken, and for the total 24-hour urine volume, the final results were expressed as mg 17-oxosteroids per 24 hours.

Standard dehydroepiandrosterone (DHA), in the diethyl ether phase, was found to obey the Beer-Lambert Law (Figure 2.5).

Recovery experiments were carried out frequently. The recovery of DHA added to water and urine and processed through the method was consistently above 80 per cent. Typical results of recovery experiments are given in Table 2.II. Replicate estimations of 17-oxosteroids were always made. When the results of duplicates differed by $\pm$ 5 per cent from the mean the test was repeated.

ESTIMATION OF PLASMA TESTOSTERONE

All patients were investigated by permission of fully informed parents and in accordance with the ethical standards of the establishment.

Peripheral venous blood (10-20 ml) was delivered immediately to a tube containing lithium heparin. The plasma was separated by centrifugation and transferred to a plain tube. The plasma was either processed immediately or stored at -15°C until assayed.

A flow diagram of the methods used for extraction, purification and quantitation of testosterone in plasma is shown in Figure 2.6.

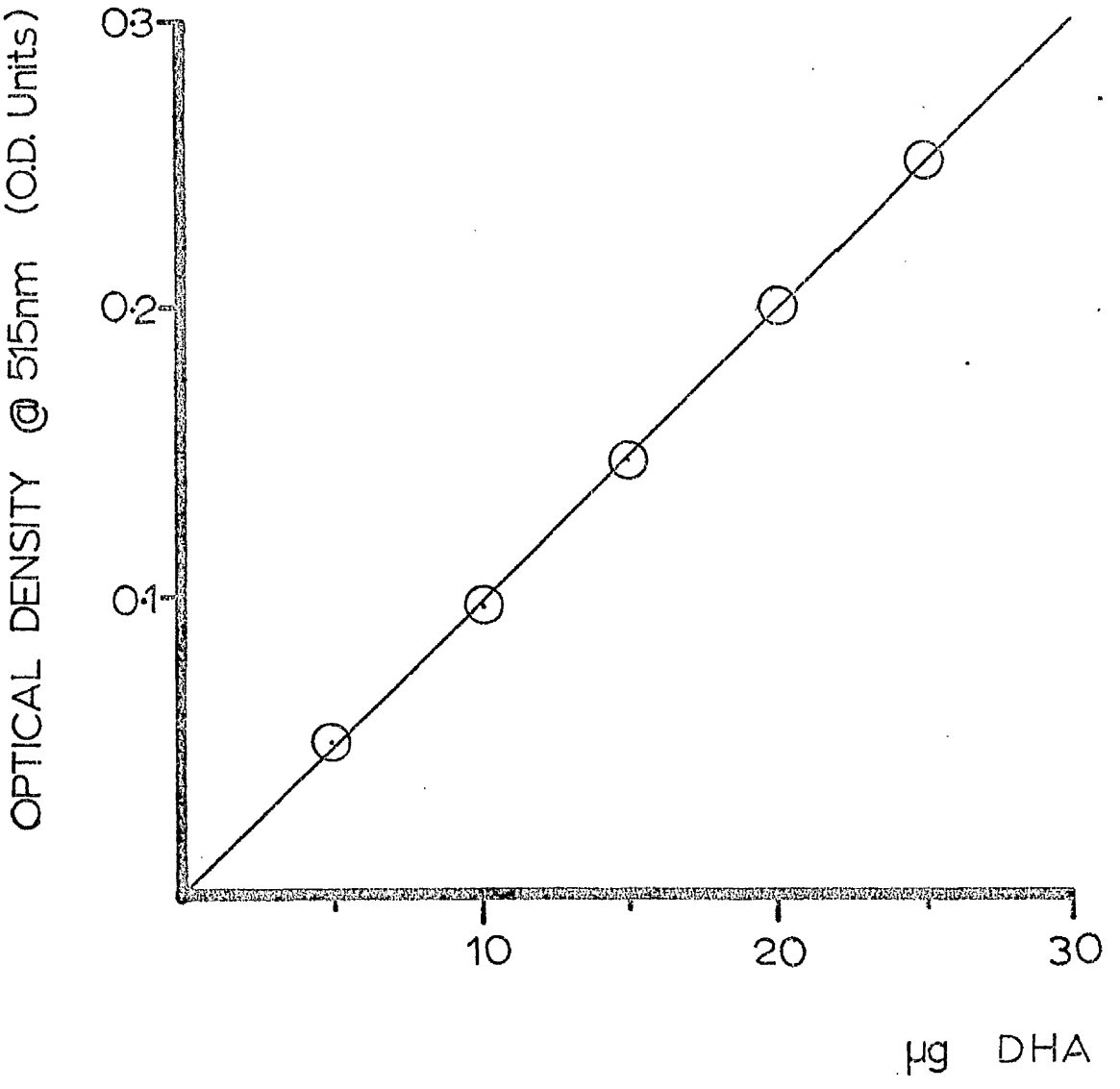


Figure 2.5 Optical density of the Zimmermann colour for DHA in the range 5-25 µg. The results were corrected for reagent blank. Note the obedience of the results to the Beer-Lambert Law.

TABLE 2.II RECOVERY OF DHA FROM WATER AND URINE.
THE COMPLETE METHOD OF ASSAY FOR 17-OXOSTEROIDS WAS USED.

Extraction was made with chloroform (10 ml).
Aliquot of 5 ml taken for the Zimmermann reaction.

Test	Wavelength (nm) 435	515	595	AC	AC-RB	Percentage Recovery of DHA
Reagent Blank	054	044	012	011	000	
	047	040	011	011		
Standard DHA (10 µg)	077	188	073	113	099	
	072	178	070	107		
Water	010	018	002	012	001	
	012	018	000	012		
Water plus DHA (20 µg)	074	184	062	116	098	97.98*
	072	168	060	102		
Urine A (adult)	174	317	146	157	143	
	356	400	142	151		
Urine A plus DHA (20 µg)	294	491	196	246	229	86.87*
	292	476	192	234		

AC = Allen correction (Allen, 1950)

RB = Reagent Blank

* Adjusted for aliquot taken for Zimmermann reaction

EXTRACTION

[1,2,6,7-^{3}H]Testosterone (S.A. 87 Ci/mM, 0.036 μCi) was added to each sample of plasma for estimation of the recovery. The plasma was made alkaline by the addition of 1 M sodium hydroxide (1 ml) to restrict the quantity of lipids likely to be extracted (O'Gata and Hirano, 1933).

After the addition of the sodium hydroxide the plasma was immediately extracted three times with diethyl ether (3 x 20 ml). The diethyl ether extract was washed with 10 per cent acetic acid (5 ml) and deionized water (5 ml). Washing with water was repeated until the extract was neutral. The neutralized ether extract was transferred to a boiling tube (50 ml) which was then placed in a water bath at 40°C. The ether was removed by blowing to dryness under a stream of nitrogen. The dried extract was dissolved in 70 per cent methanol (10 ml) and partitioned against hexane (2 x 10 ml) (Oertel, 1961). The lower aqueous methanol layer was removed and blown to dryness under a stream of nitrogen as before.

THIN LAYER CHROMATOGRAPHY OF FREE STEROIDS

The dried extract was quantitatively applied to the origin of a silica thin layer using dichloromethane (3 x 20 μl) and methanol (2 x 20 μl) as vehicles. The chromatogram was developed in the solvent system, benzene:methanol (85:15, v/v) (Guerra-Garcia *et al.*, 1963) for two, one-hour periods, allowing the plate to dry in air before the second development. In this solvent system the R_f of testosterone is 0.58. The R_s (testosterone) values of standard steroids and their sulphate and glucuronoside esters are given in Table 2.III. The silica over the radioactive area, representing the ^{3}H-testosterone initially added to the plasma, and located by radiochromatographic scanning, was removed and eluted first with dichloromethane (3 x 2 ml) and then methanol (3 x 2 ml).

The eluate was collected in a conical centrifuge tube (10 ml), reduced to small volume under nitrogen, and applied quantitatively as a spot to the

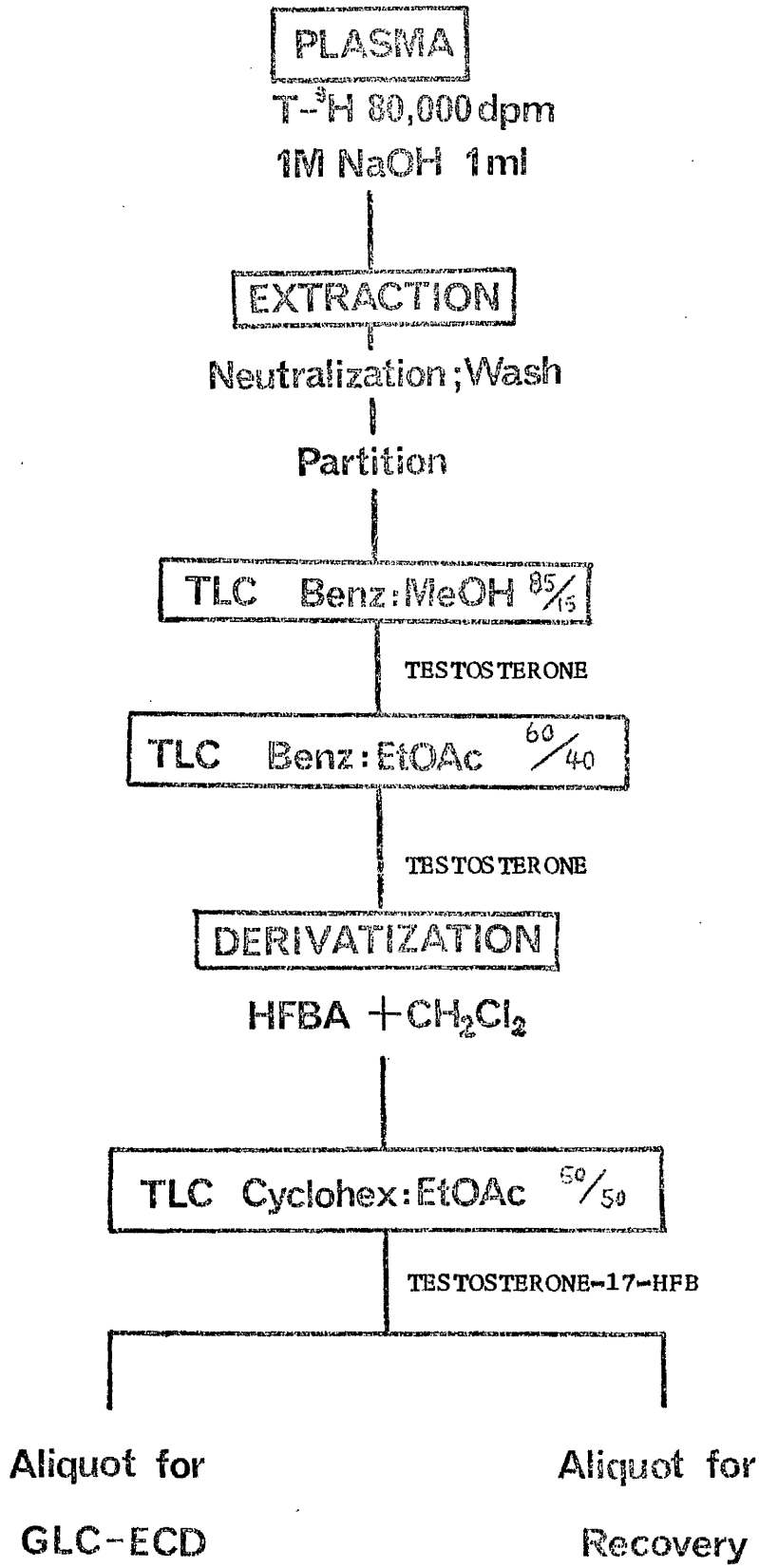


Figure 2.6 Flow diagram of the procedures used in the estimation of plasma testosterone concentration.

TABLE 2.III SEPARATION OF STEROIDS AND STEROID CONJUGATES BY THIN LAYER CHROMATOGRAPHY IN BENZENE:METHANOL (85:15, v/v)

	R_s value*
Testosterone β-D-glucuronoside	0.01
Dehydroepiandrosterone glucuronoside	0.01
Androsterone sulphate	0.07
Dehydroepiandrosterone sulphate	0.08
Oestriol	0.23
Cortisol	0.46
11β-Hydroxytestosterone	0.49
5α-Androstane-3β,17β-diol	0.55
5β-Androstane-3α,17β-diol	0.67
5α-Androstane-3α,17β-diol	0.75
17α-Hydroxypregnenolone	0.76
17α-Hydroxyprogesterone	0.79
11β-Hydroxyaetiocholanolone	0.80
11β-Hydroxyandrosterone	0.88
17β-Oestradiol	0.88
Testosterone	1.00
11-Oxoandrosterone	1.01
Epitestosterone	1.03
11-Oxoaetiocholanolone	1.07
Epiandrosterone	1.13
Dehydroepiandrosterone	1.14
5β-Dihydrotestosterone	1.17
Δ^5-Pregnenolone	1.18
Aetiocholanolone	1.18
5α-Dihydrotestosterone	1.19
Androsterone	1.21
Δ^4-Androstenedione	1.31
Oestrone	1.32
Progesterone	1.38
5α-Androstanedione	1.41

Development 2 x 1 hour

* s = testosterone R_f 0.58

origin of a second silica thin layer using ethyl acetate (4 x 10 µl) and methanol (2 x 10 µl) as vehicles. The chromatogram was developed in the solvent system, benzene:ethyl acetate (6:4, v/v) (Korenman et al., 1963) for two, one-hour periods as before. R_s values (relative to testosterone, R_f 0.30) of standard steroids developed in this solvent system are given in Table 2.IV. The silica over the radioactive testosterone area as located by radiochromatographic scanning, was removed and eluted with ethyl acetate (3 x 2 ml) and dichloromethane (3 x 2 ml). The eluate was dried under a stream of nitrogen as above and esterified with heptafluorobutyric anhydride (page 71).

ESTERIFICATION OF STEROIDS

The low level of testosterone in urine and plasma of children demands sensitive methods for its detection. Clark and Wotiz (1963) first observed that heptafluorobutyrate derivatives of steroids exhibited a high electron affinity, due to the introduction of 7 fluorine atoms into the steroid molecule. Nakagawa et al. (1966) investigated several halo-alkyl esters of testosterone with regard to their sensitivity to electron capture detection. They found testosterone mono-heptafluorobutyrate one of the most sensitive, although the di-heptafluorobutyrate, monochlorodifluoroacetate and perfluoro-octanoate esters showed an even higher electron affinity. Van der Molen et al. (1967), using the mono-heptafluorobutyrate ester of testosterone for the detection of testosterone in plasma, were able to detect its presence in nanogram (ng) and picogram (pg) quantities. Plasma extracts and steroid standards were treated with heptafluorobutyric anhydride using a modified method for esterification.

Preparation of Standard Testosterone-17-heptafluorobutyrate

Esterification of testosterone may occur at C17 and also at C3 by enolization of the 3-keto-group. The reaction of testosterone with heptafluorobutyric anhydride under different conditions gives high yields of

TABLE 2.IV SEPARATION OF STEROIDS BY THIN LAYER CHROMATOGRAPHY IN BENZENE:ETHYL ACETATE (6:4, v/v)

Free steroid	R_s value*
11β-Hydroxyaetiocholanolone	0.61
11-Oxoaetiocholanolone	0.62
11β-Hydroxyandrosterone	0.74
11-Oxoandrosterone	0.74
Testosterone	1.00
Epitestosterone	1.06
Δ^5-Pregnenolone	1.24
5β-Dihydrotestosterone	1.28
Epiandrosterone	1.30
Dehydroepiandrosterone	1.45
Androsterone	1.47
Aetiocholanolone	1.50
5α-Dihydrotestosterone	1.50
17β-Oestradiol	1.63
Δ^4-Androstenedione	1.65

Development 2 x 1 hour

* s = testosterone R_f 0.30

testosterone-17-mono-heptafluorobutyrate and two isomeric enol esters, the diheptafluorobutyrates of androst-2,4-diene-3β,17β-diol and androst-3,5-diene-3β,17β-diol (Devaux and Horning, 1969). As both the diheptafluorobutyrates are reported to be unstable on silica gel thin layer chromatography (Van der Molen et al., 1967; Devaux and Horning, 1969) reaction conditions were used to form testosterone-17-heptafluorobutyrate in high yield.

Testosterone (1 mg) was dissolved in dichloromethane (1 ml) and heptafluorobutyric anhydride (50 µl) added. After thorough mixing on a Vortex mixer, the reactants were allowed to stand at room temperature for 30 minutes. Excess reagent was removed by evaporation to dryness at room temperature under a stream of nitrogen. [1,2,6,7-^{3}H]Testosterone (S.A. 87 Ci/mM; 20 nCi) was added to the standard testosterone, prior to esterification, for recovery purposes. The dried esterified testosterone was quantitatively applied to the origin of a silica thin layer using ethyl acetate (5 x 20 µl) and the chromatogram developed in cyclohexane:ethyl acetate (1:1, v/v). A two-dimensional development was used, each development period being 55 minutes. The TLC plate was allowed to dry after the first development before being turned through 90° for the second development. The location of the UV-absorbing steroids is shown in Figure 2.7. The silica area containing testosterone-17-heptafluorobutyrate was scraped from the plate and eluted with ethyl acetate (3 x 2 ml) and acetone (3 x 2 ml).

The solvent was removed by evaporation under a stream of nitrogen. The ester was dissolved in ethyl acetate (1 ml). An aliquot (0.1 ml) was taken for liquid scintillation counting of radioactivity and from this count the percentage conversion to the mono-heptafluorobutyrate was calculated. A conversion of 85 per cent of testosterone to testosterone-17-heptafluorobutyrat (T-HFB) was consistently achieved by this method. Calculated dilutions of the ester were made in ethyl acetate such that 1 µl contained 1 µg T-HFB for FID and 1 µl contained 1 ng T-HFB for ECD.

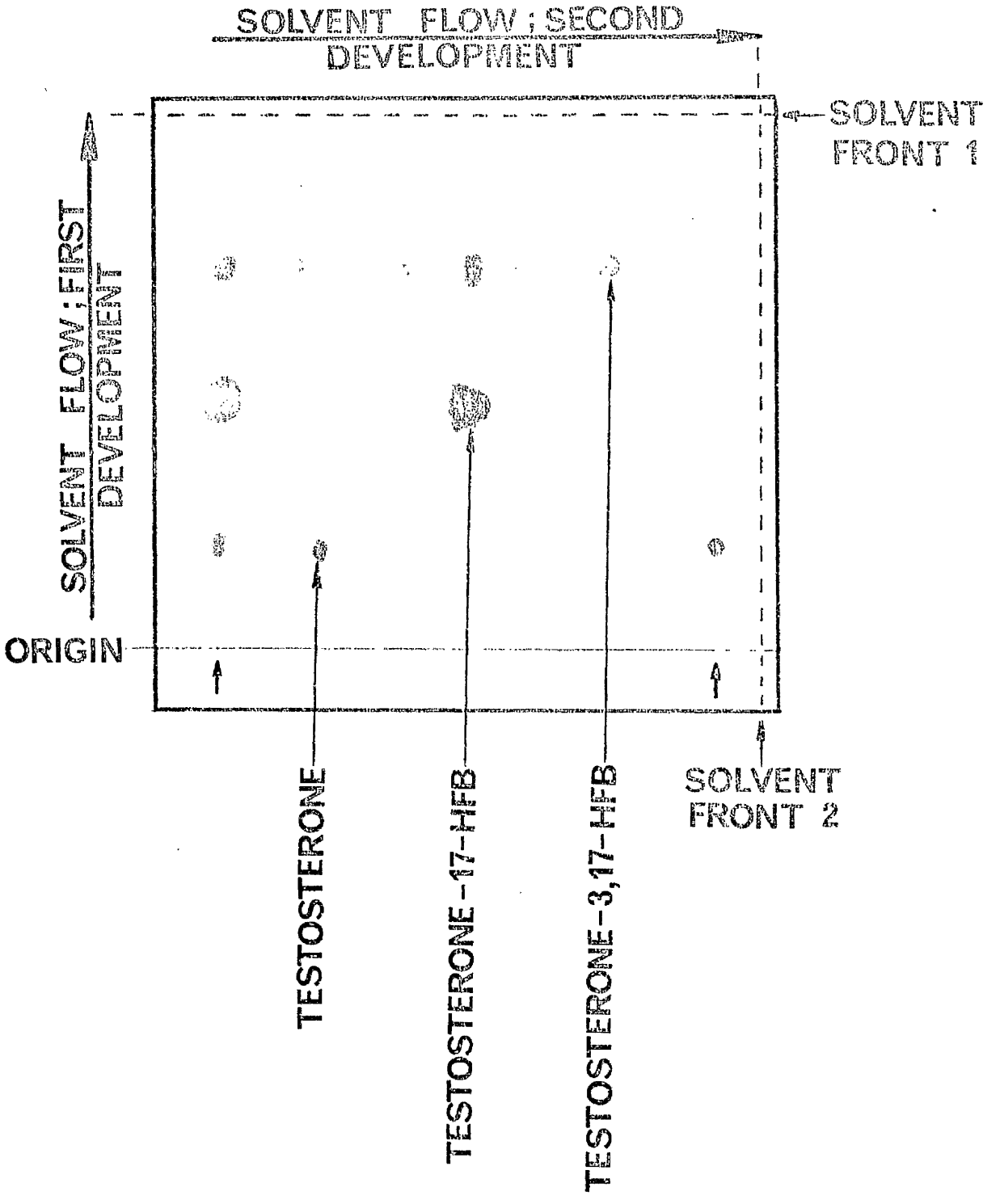


Figure 2.7 Two-dimensional TLC separation of testosterone heptafluorobutyrates in cyclohexane:ethyl acetate (1:1, v/v). Development for 2 x 55 minutes. Note breakdown of testosterone-3,17-HFB on second development.

A single peak was obtained by GLC analysis of the T-HFB (Figure 2.8).

Preparation of Standard Androsterone-3-heptafluorobutyrate

Esterification of androsterone only occurs at the hydroxyl on the C3 position. Androsterone-3-heptafluorobutyrate was therefore prepared under the same reaction conditions as for testosterone-17-heptafluorobutyrate.

Androsterone (1 mg) was dissolved in dichloromethane (1 ml) to which was added heptafluorobutyric anhydride (50 µl). After thorough mixing on a Vortex mixer, the reactants were allowed to stand at room temperature for 30 minutes. Excess reagent was removed by evaporation to dryness at room temperature under a stream of nitrogen. [1α,2α-^{3}H]Androsterone (S.A. 40 Ci/m 20 nCi) was added to the standard prior to esterification, for recovery purposes. Partition of ^{3}H-androsterone between n-hexane and 70 per cent aqueous methanol (1:1, v/v) gave a quantitative recovery of radioactivity from the aqueous methanol phase. When partition between the above solvents was repeated after esterification 99.8 per cent of the original radioactivity was recovered in the hexane phase indicating a quantitative conversion of androsterone to androsterone-3-heptafluorobutyrate.

This phase was then reduced to a small volume under nitrogen and applied quantitatively to the origin of a silica thin layer using ethyl acetate (2 x 20 µl) and acetone (2 x 20 µl). The TLC plate was then developed in cyclohexane:ethyl acetate (1:1, v/v) for one hour. Following radiochromatographic scanning the silica on the radioactive area was scraped off and eluted with ethyl acetate (3 x 2 ml) and acetone (3 x 2 ml). The eluate was blown to dryness under a stream of nitrogen and the ester redissolved in ethyl acetate (1 ml). An aliquot (0.1 ml) was taken for liquid scintillation counting of radioactivity and from this count the percentage conversion to androsterone-3-heptafluorobutyrate was calculated. A conversion of 90 per cent of androsterone to androsterone-3-heptafluorobutyrate (A-HFB) was consistently achieved by this method. Calculated dilutions of the ester were made in ethyl acetate such that 1 µl contained 1 µg A-HFB for FID and

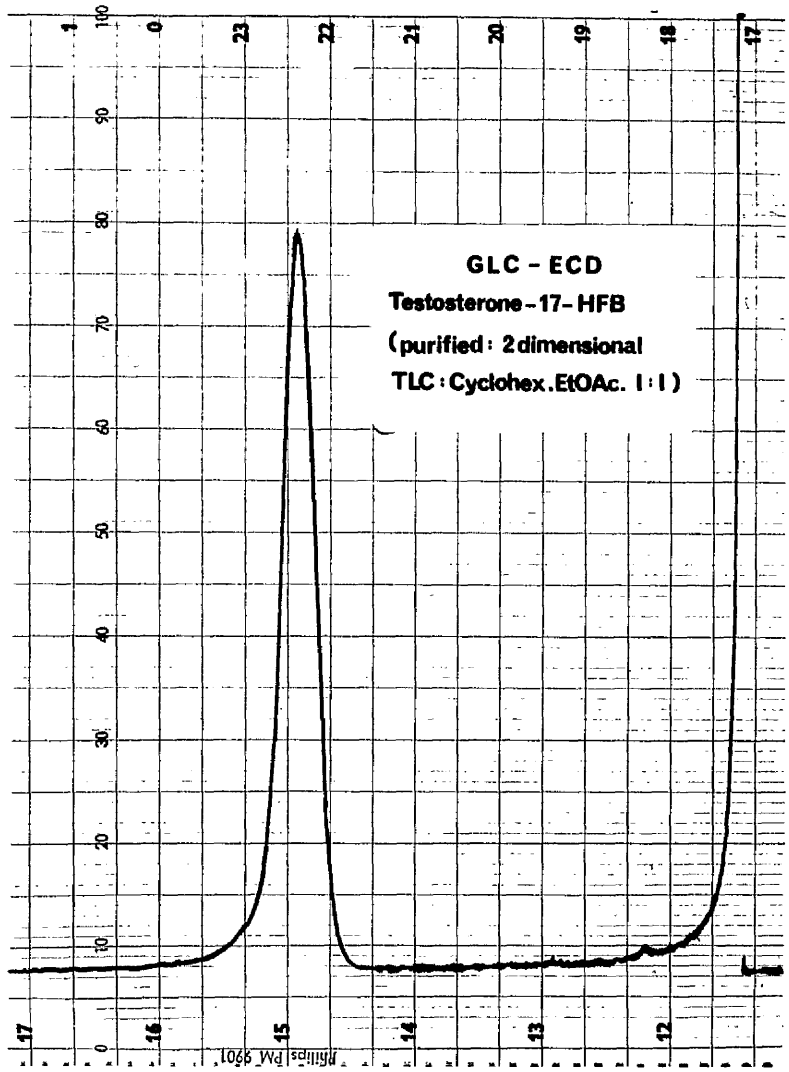


Figure 2.8 Gas liquid chromatographic analysis with electron capture detection of testosterone-17-HFB following purification on a two-dimensional TLC separation in cyclohexane:ethyl acetate (1:1, v/v). This and subsequent tracings have been overlined in black ink to facilitate photography.

1 µl contained 1 ng A-HFB for ECD. Gas liquid chromatography of standard androsterone esterified by this method gave a single peak.

Esterification of Plasma Extracts

The dried residues obtained from the TLC plates developed in benzene:ethyl acetate (6:4, v/v) were placed in a vacuum desiccator overnight. The residues were dissolved in dichloromethane (1 ml) and esterified with heptafluorobutyric anhydride (50 µl) at room temperature for 30 minutes. Excess reagent was removed by evaporation to dryness at room temperature under a stream of nitrogen. The dried residue was applied to the origin of a silica thin layer using ethyl acetate (2 x 20 µl) and acetone (2 x 20 µl). The TLC plate was developed in the solvent system cyclohexane:ethyl acetate (1:1, v/v) for one hour. R_f values of free standards and esterified steroids are given in Table 2.V. The TLC plate was then scanned and the area of silica under the peak of radioactivity (corresponding in R_f with that of standard testosterone-17-HFB) was removed and eluted with ethyl acetate (3 x 2 ml) and then acetone (3 x 2 ml). The extract was dried under nitrogen in a water bath at 40°C. The dried extract was redissolved in ethyl acetate (dried over anhydrous magnesium sulphate) (50 µl). An aliquot (5 µl) was then removed for liquid scintillation counting to permit assessment of the overall recovery rate and an aliquot injected into the GLC-ECD system (page 76).

GAS LIQUID CHROMATOGRAPHY

Hartman and Wotiz (1963) have found neopentyl glycol sebacate (N.G.Seb.) a suitable liquid phase for the separation of the trimethyl-silyl ether derivatives of androgen and progesterone metabolites. After preliminary experiments it was decided to use the temperature stabilized form of this polyester in the present investigation to allow the use of higher operating temperatures, so obviating long retention times.

TABLE 2.V SEPARATION OF STANDARD STEROIDS (FREE AND HEPTAFLUOROBUTYRATE ESTERS) IN CYCLOHEXANE:ETHYL ACETATE (1:1, v/v)

Free Steroid	R_s*	Heptafluqrobutyrate Ester (HFB)	R_s*
11β-Hydroxyandrosterone	0.30	3α-Hydroxyl HFB	1.09
		3α,11β-Hydroxyl HFB	1.26
Testosterone	0.40	17β-Hydroxyl HFB	1.00
		3-enol,17β-Hydroxyl HFB	1.62
Epitestosterone	0.43	17α-Hydroxyl HFB	1.02
		3-enol,17α-Hydroxyl HFB	1.74
Epiandrosterone	0.52	3β-Hydroxyl HFB	1.22
5β-Dihydrotestosterone	0.55	17β-Hydroxyl HFB	1.01
Dehydroepiandrosterone	0.59	3β-Hydroxyl HFB	1.01
11-Oxoandrosterone	0.59	3α-Hydroxyl HFB	1.29
Δ^5-Pregnenolone	0.62	3β-Hydroxyl HFB	1.32

Development for 1 hour

* R_s relative to testosterone-17-HFB (R_f 0.45)

Preliminary Silanization of the Column

Prior to packing, the empty glass column (5 ft, 4 mm I.D.) was rinsed with concentrated hydrochloric acid, washed with deionized water and then silanized with 5 per cent (v/v) dimethyldichlorosilane in toluene for 15 minutes. The column was emptied, washed several times with methanol and thoroughly dried at 100-110°C for one hour. This procedure is recommended by Horning (1968).

Preparation of Column Packing Material

Gas Chrom Q, 100-120 mesh (Phase Separations Ltd) was used as the support without further purification. A solution of N.G.Seb. (0.5 per cent, wt/v), was prepared by dissolving N.G.Seb. (0.5 g) in chloroform (100 ml). This concentration of liquid phase was routinely used to give a 1 per cent coating of the Gas Chrom Q. The relationship between the concentration of the liquid phase in solution and the percentage of liquid phase coating the support material is fortuitous. However, the procedure was recommended by Dr T. Simpson of Torry Research Station, Aberdeen (personal communication).

The liquid phase (100 ml) was slowly added to Gas Chrom Q (15 g) in vacuo by the filtration method. The flask contents were slowly swirled so as to dislodge trapped air and to facilitate an even coating of the support. The vacuum was then broken and the contents quickly filtered through a Buchner system. Suction was continued for five minutes to remove the chloroform. The coated support, retained in the filter funnel, was then transferred, without washing, onto a 20 cm diameter watch glass. It was then dried in air at 37°C.

Packing and Conditioning the Column

A small glass filter funnel was attached by tubing to the injection end of the column. The coated support was then added in small amounts to the funnel and filling of the column facilitated by suction applied to the detector-end of the column. Gentle vibration was applied along the length

of the column using a hand-vibrator (Phase Separations Ltd). The injection end of the column was not completely filled so as to accommodate the flash heater. A silanized glass wool plug was tamped into position at the injection end of the column.

The packed column was placed in the column oven of the GLC system and conditioned at 220°C whilst a slow flow of oxygen-free nitrogen, as carrier gas, was maintained through the column. The carrier gas flow rate was slowly increased to 50 ml per minute. Conditioning in this environment was continued overnight to remove excess liquid phase from the column, before coupling the column to the detector.

Operating Conditions

The separating powers of the column were preliminarily determined using flame ionization detection (FID). Urine and plasma extracts were quantitatively analysed for testosterone using electron capture detection (ECD). Recovery of radioactivity which had been initially added to the biological material permitted assessment of recovery.

The column oven was operated at 190°C, the injection heater at 210°C and the detector oven at 250°C when using ECD. Samples were injected into the column using an S.G.E. 10 µl syringe fitted with an 11.5 cm needle.

Linearity of the Detector

The response for both testosterone-17-heptafluorobutyrate and the internal standard, androsterone-3-heptafluorobutyrate, was proportional to the amount injected (Figure 2.9). An internal standard (androsterone-3-heptafluorobutyrate) was injected with each sample.

Linearity of detector response to varying amounts of steroid derivative was determined

(a) by using peak height only,

(b) by using peak area determined from the product of the peak height and the width of the peak at half peak height,

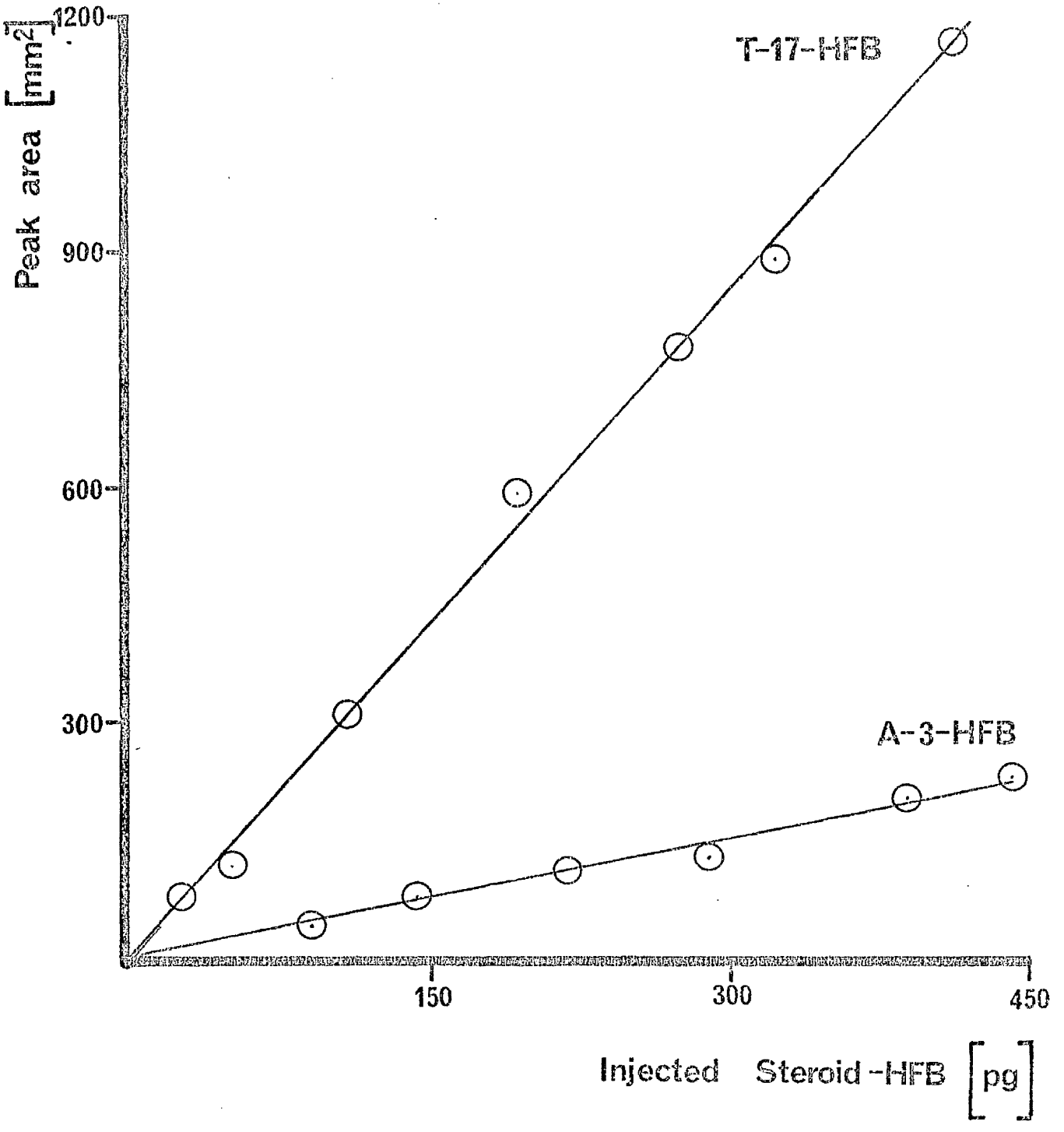


Figure 2.9 Electron capture detector response (peak areas) to varying amounts of testosterone-17-HFB and androsterone-3-HFB.

(c) by using peak area determined by triangulation, the area being determined by halving the product of the peak height and the peak width at the baseline.

The linearity of detection using all three modes of quantitation is shown in Figure 2.10. Quantitation of peaks using the peak area determined from the product of the peak height and the width of the peak at half peak height was used routinely throughout this investigation.

QUANTITATION OF TESTOSTERONE IN PLASMA

Standard amounts of testosterone-17-heptafluorobutyrate and androsterone-3-heptafluorobutyrate were injected daily prior to sample analysis. A known amount of androsterone-HFB was injected as an internal standard together with 5-8 µl of the esterified plasma extract. Quantitation of the testosterone-17-HFB in the test samples was made by comparison of the ratios of the peak area of the standard testosterone-17-HFB to the peak area of the standard androsterone-HFB with the peak area of testosterone-17-HFB in the plasma extract to the peak area of androsterone-3-HFB added as the internal standard. Correction was made for the aliquot injected, and for the experimental recovery as estimated by the recovery of ^{3}H-testosterone added initially to each sample. The mass of ^{3}H-testosterone-HFB injected into the GLC system with the plasma extract was deducted from the final quantitation. The results were adjusted to 100 ml plasma volumes. An example of the estimation of testosterone in a plasma sample is given in Figure 2.11 and the accompanying explanatory text.

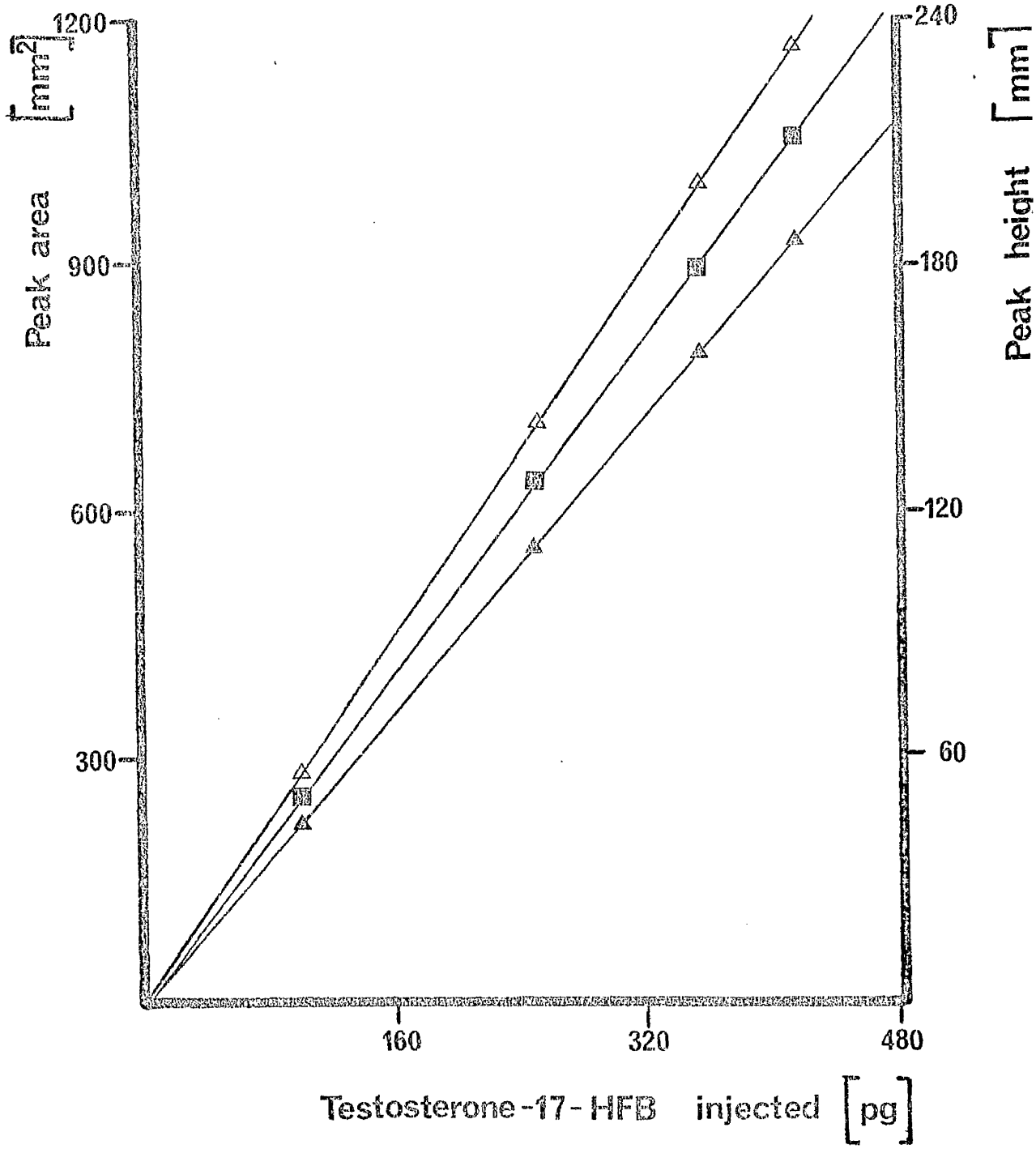


Figure 2.10 Linearity of electron capture detector response determined from peak height and peak area measurements.

KEY:

—△— Peak height

—▣— Peak area by triangulation

—△— Peak area (width at half peak height x peak height)

A

B

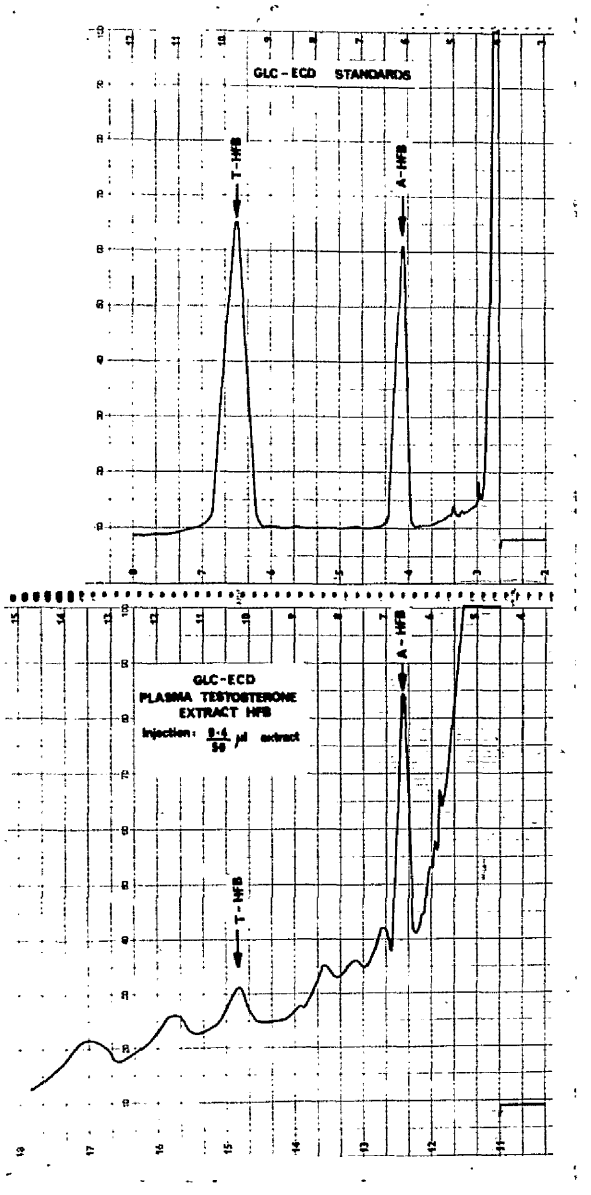


Figure 2.11 The estimation of plasma testosterone concentration. GLC-ECD tracing of a plasma extract from a patient under investigation.

A. Standard trace

12.5 ng A-HFB gives a peak area of 699 mm^2.

5 ng T-HFB gives a peak area of 1,477 mm^2.

B. Plasma extract

12.5 ng of the internal standard A-HFB gives a peak area of 638 mm^2. Since this peak area differs from that obtained in the standard trace (Figure 2.11A) the corresponding peak area for 5 ng T-HFB in the second trace is therefore:

$$\frac{1,477 \times 638}{699} \ mm^2$$

But the peak area of native T-HFB in the second trace is 210 mm^2. Therefore there are

$\frac{699 \times 5 \times 210}{1,477 \times 638}$ ng T-HFB in the sample injected (9.4 µl of 50 µl)

and $\frac{699 \times 5 \times 210 \times 50}{1,477 \times 638 \times 9.4}$ ng T-HFB in the plasma residue.

But $^1/10$ of the residue was removed previously for calculation of recovery. This recovery was 29.42 per cent* Therefore if the recovery of the native steroid is equivalent to the recovery of ^{3}H-T added initially to the plasma, then there were

$\frac{699 \times 5 \times 210 \times 50 \times 100 \times 10}{1,477 \times 638 \times 9.4 \times 29.42 \times 9}$ ng of testosterone in the plasma extract (10.5 ml)

Therefore in 100 ml of plasma there are

$\frac{699 \times 5 \times 210 \times 50 \times 100 \times 10 \times 100}{1,477 \times 638 \times 9.4 \times 29.42 \times 9 \times 10.5}$ ng of testosterone

ie 149 ng of testosterone per 100 ml.

* Low recovery. Normal recovery range 35-50 per cent.

ESTIMATION OF URINARY TESTOSTERONE

Horton et al. (1963) and Dulmanis et al. (1964) have shown that testosterone is present in urine in the free form, conjugated as the glucuronoside and in a pH 1 hydrolysable form (probably as a sulphate).

As the quantity of urinary testosterone in prepubertal children is very small (and the excretion of testosterone in the patients selected was likely to be below normal) it was decided that a single, 'near total' extraction of all urinary steroid conjugates would give more workable levels of testosterone for subsequent purification and detection than three separate extracts obtained from a fractionated extraction procedure.

HYDROLYSIS OF TESTOSTERONE GLUCURONOSIDE

Some authors have extracted free testosterone from urine prior to hydrolysis (Camacho and Migeon, 1963; Sandberg et al., 1964; Van der Molen et al., 1966) but most methods for the estimation of urinary testosterone employ β-glucuronidase hydrolysis without preliminary extraction (Vermeulen and Verplanke, 1963; Futterweit et al., 1963; Korenman et al., 1963; Korenman et al., 1964; Brooks, 1964; Schubert and Frankenberg, 1964; Ibayashi et al., 1964). In this investigation free testosterone was not extracted before hydrolysis.

The hydrolysis of urinary testosterone glucuronoside was effected by adding β-glucuronidase (75,000 F.u.) to urine (100 ml) at pH 4.6 and incubating the mixture at 37°C for 72 hours. The pH was maintained by the addition of 2 M acetate buffer (272 g sodium acetate trihydrate and 120 ml glacial acetic acid diluted to 1 litre with deionized water) (10 ml). No attempt was made to inhibit the sulphatase activity of the β-glucuronidase enzyme preparation.

The hydrolysed urine was filtered through Whatman GF/A glass fibre paper (H. Reeve Angel & Co. Ltd) into a separating flask (500 ml). /~1,2,6,7-^{3}H~/Testosterone (S.A. 87 Ci/mM, 36 nCi) was added to the filtrate

for recovery purposes. The filtrate was extracted with diethyl ether (4 x 100 ml) and the extract reduced to approximately 200 ml under a stream of nitrogen in a water bath at 40°C. The extract was washed with 5 M sodium hydroxide (10 ml) and deionized water (20 ml) until the water washings were neutral. The extract was then dried by filtration through anhydrous sodium sulphate. The recovery of ^{3}H-testosterone (added initially to urine samples) indicated that at this stage the recovery of free testosterone was 99.4 per cent. Thereafter samples were subjected to thin layer chromatography as for the plasma extract (page 85).

Evaluation of the Hydrolysis Method

The potency of the β-glucuronidase preparation used throughout this study was estimated using phenolphthalein glucuronoside as substrate. The enzyme preparation was also known to have significant sulphatase activity. The sulphatase activity was estimated using radioactively labelled dehydroepiandrosterone sulphate (^{3}H-DHAS) as substrate. Incubation studies were carried out at pH 4.6 in aqueous solution, the pH being maintained with 2 M acetate buffer. Inhibition of sulphatase in control samples was achieved by addition of potassium dihydrogen phosphate (500 mg) to the incubation mixture. Free radioactively labelled dehydroepiandrosterone released from ^{3}H-DHAS was extracted with chloroform (2 x 50 ml). As partition of ^{3}H-DHAS between water and chloroform resulted in 3.4 per cent of the radioactivity being recovered from the organic phase, control values were subtracted from the test samples. Results are shown in Figure 2.12.

The effect of increasing concentration of the β-glucuronidase preparation on sulphatase activity is shown in Figure 2.13. Here unlabelled testosterone sulphate was used rather than DHAS-^{3}H and it was assumed that sulphatase was equally active for both the 3- and 17-sulphate. Testosterone sulphate (100 µg) was added to 50 ml aliquots of water. A series of 12 flasks were incubated at 37°C for 72 hours, with varying amounts of

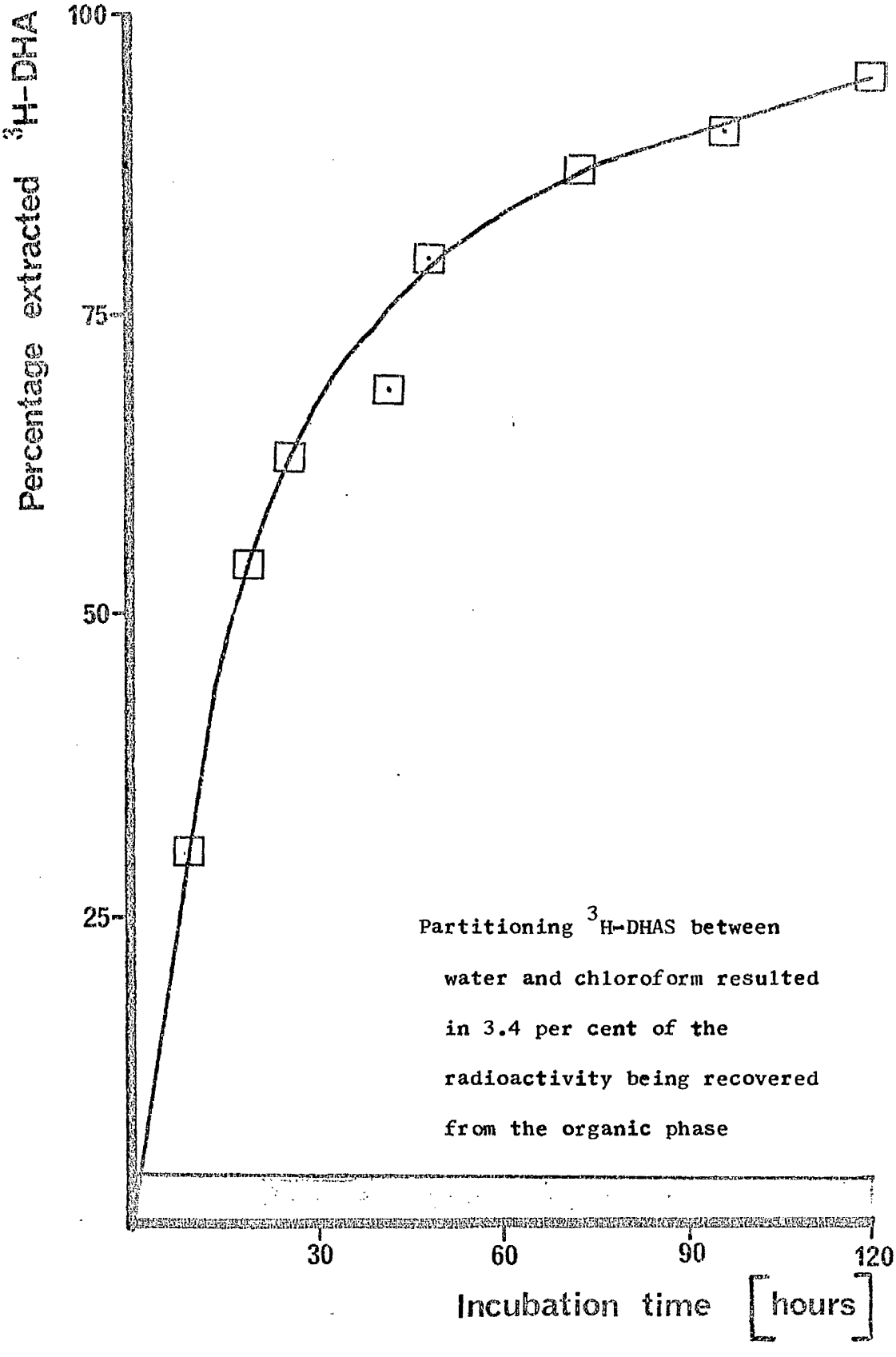


Figure 2.12 The recovery of ^{3}H-DHA from ^{3}H-DHAS added to aliquots of water (50 ml) and incubated with β-glucuronidase (750 F.u. per ml) for varying lengths of time.

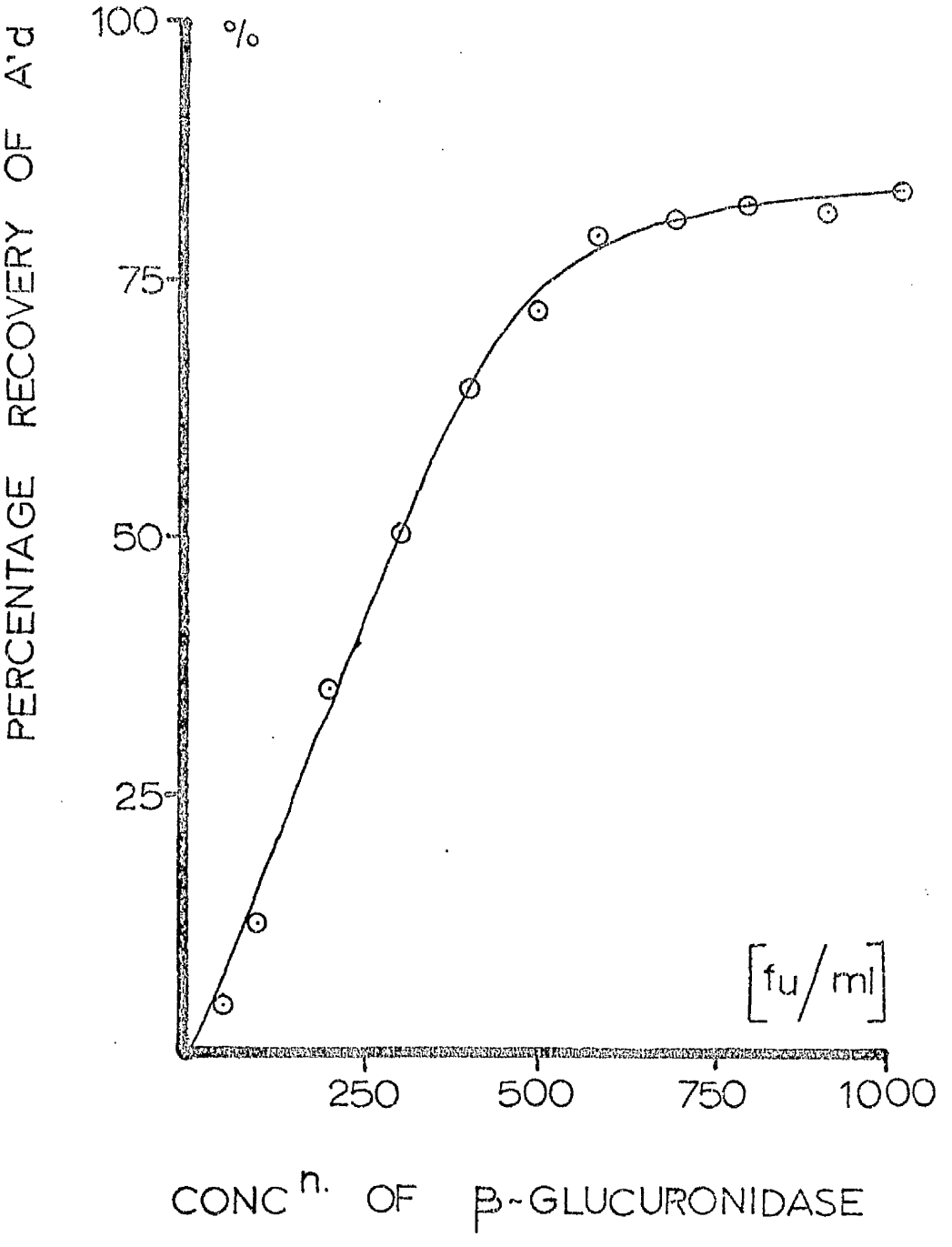


Figure 2.13 The recovery of androstenedione from testosterone sulphate (100 µg) added to aliquots of water (50 ml) and incubated with varying concentrations of β-glucuronidase for 72 hours at 37°C. Androstenedione formed by the oxidation of testosterone was assayed by the Zimmermann reaction.

β-glucuronidase powder. A control flask was incubated as above but with potassium dihydrogen phosphate (500 mg) to prevent sulphatase activity. Free testosterone was extracted with dichloromethane (2 x 50 ml). The extract was dried under a stream of nitrogen in a water bath at 40°C and redissolved in acetone (500 µl). Subsequently, testosterone was oxidised with Kiliani reagent (500 µl) (Kiliani and Mark, 1901) at room temperature for 20 minutes. Water (2.5 ml) was added and the Δ^4-androstenedione so formed was extracted with benzene:chloroform (6:1, v/v) (2 x 2 ml). Assay of androstenedione was by the Zimmermann reaction using androstenedione (50 µg) as the standard. From Figure 2.13 it will be seen that maximum hydrolysis is achieved using more than 30,000 F.u. of β-glucuronidase per 50 ml (600 F.u. per ml). Routinely 750 F.u. β-glucuronidase per ml were employed for the hydrolysis of urine samples.

The effect of increasing concentrations of β-glucuronidase on glucuronidase activity was assayed using unlabelled testosterone glucuronoside as substrate. Testosterone glucuronoside (100 µg) was added to 50 ml aliquots of water. A series of 12 flasks were incubated at 37°C for 72 hours with varying amounts of β-glucuronidase. A control flask was incubated as above but without β-glucuronidase. Testosterone was extracted with dichloromethane (2 x 50 ml). The extract was dried under nitrogen in a water bath at 40°C and oxidised by the Kiliani reaction. The androstenedione so formed was extracted with benzene:chloroform (6:1, v/v) (2 x 2 ml) and assayed by the Zimmermann reaction using androstenedione (50 µg) as standard. From Figure 2.14 it will be seen that maximum hydrolysis is achieved using more than 20,000 F.u. of β-glucuronidase per 50 ml (400 F.u. per ml). Routinely 750 F.u. per ml were used for the hydrolysis of urine samples.

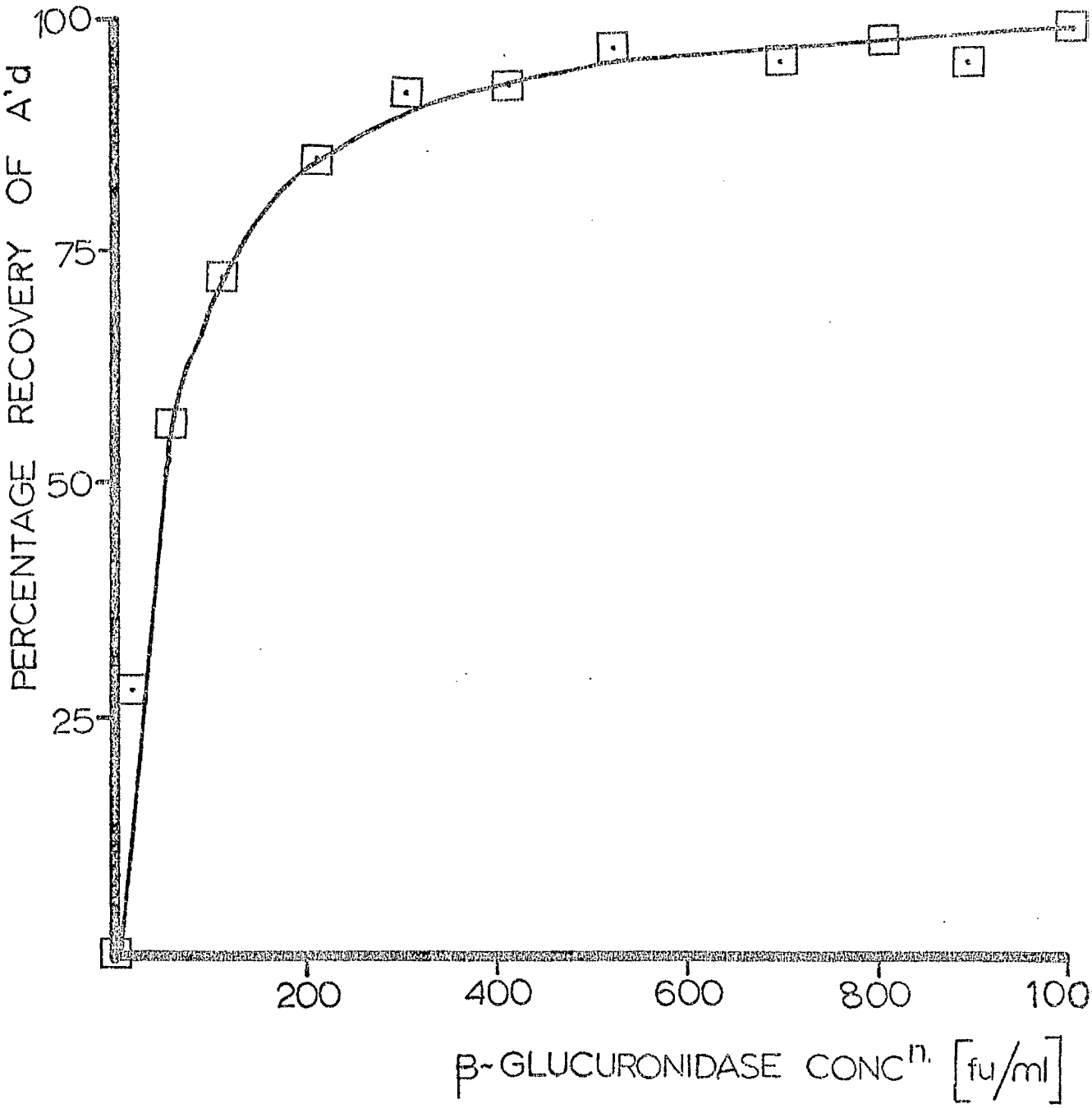


Figure 2.14 The recovery of androstenedione from testosterone glucuronoside (100 μg) added to aliquots of water (50 ml) and incubated with varying concentrations of β-glucuronidase for 72 hours at 37°C. Androstenedione formed by the oxidation of testosterone was assayed by the Zimmermann reaction.

Purification of Urine Extracts

Following extraction the dried residues were applied to the origin of a silica thin layer using dichloromethane (3 x 20 µl) and methanol (2 x 20 µl) The chromatogram was developed in the solvent system benzene:methanol (85:15,v/v) for two, one-hour periods, allowing the plate to dry in air before the second development. R_s values of standard steroids in this system are shown in Table 2.III. The radioactive area, representing ^{3}H-testosterone added initially to the urine, was located by radiochromatographic scanning, scraped from the plate, and transferred to a 'thimble vac' under vacuum. The steroid was eluted from the silica with dichloromethane (3 x 2 ml) and methanol (3 x 2 ml) into a conical centrifuge tube (10 ml). The purified extract was reduced to small volume under nitrogen in a water bath at 40°C, and applied quantitatively to a silica thin layer using ethyl acetate (4 x 10 µl) and methanol (2 x 10 µl) as solvents. The chromatogram was then developed in the solvent system benzene:ethyl acetate (6:4, v/v) for two, one-hour periods, allowing the plate to dry before the second development. The R_s values of standard steroids in this system are shown in Table 2.IV. The radioactive area, representing ^{3}H-testosterone added initially to the urine, was located by radiochromatographic scanning and again the silica over the area was transferred to a 'thimble vac'. The steroid material was eluted from the silica with ethyl acetate (3 x 2 ml) and dichloromethane (3 x 2 ml) into a conical centrifuge tube (10 ml). The purified extract was dried under nitrogen in a water bath at 40°C and placed in a vacuum desiccator for one hour.

The dried residue was subsequently dissolved in dichloromethane (1 ml) and esterified with heptafluorobutyric anhydride (50 µl) at room temperature for 30 minutes. Excess reagent was removed under a stream of nitrogen at room temperature. The dried esters were then applied to a silica thin layer plate using ethyl acetate (2 x 20 µl) and acetone (2 x 20 µl) and the thin

layer plate was developed in the solvent system cyclohexane:ethyl acetate (1:1, v/v) for one hour. R_s values of standard steroids in this system are shown in Table 2.V. The thin layer plate was scanned using a radiochromatogram scanner and the area of the peak corresponding to standard tritiated testosterone-17-HFB was located and the silica of that area transferred to a 'thimble vac'. The steroid was eluted from the silica with ethyl acetate (3 x 2 ml) and then acetone (3 x 2 ml) into a conical centrifuge tube (10 ml) and the extract dried under nitrogen in a water bath at 40°C. The extract was redissolved in ethyl acetate (dried over anhydrous magnesium sulphate) (50 µl). An aliquot (5 µl) was then removed for liquid scintillation counting to permit assessment of the overall recovery.

QUANTITATION OF TESTOSTERONE IN URINE

Standard amounts of testosterone-17-heptafluorobutyrate and androsterone-3-heptafluorobutyrate were injected daily prior to sample analysis. A known amount of androsterone-HFB was injected as an internal standard together with 5-8 µl of the esterified urine extract. Quantitation of the testosterone-17-HFB in the test samples was made by comparison of the ratios of the peak area of the standard testosterone-17-HFB to the peak area of the standard androsterone-HFB with the peak area of testosterone-17-HFB in the urine extract to the peak area of androsterone-3-HFB added as the internal standard. Correction was made for the aliquot injected, and for the experimental recovery as estimated by the recovery of ^{3}H-testosterone added initially to each sample. The mass of ^{3}H-testosterone-HFB injected into the GLC system with the urine extract was deducted when significant. The results were corrected to the total 24-hour urine volume. An example of the estimation of testosterone in a urine sample is given in Figure 2.15 and the accompanying text.

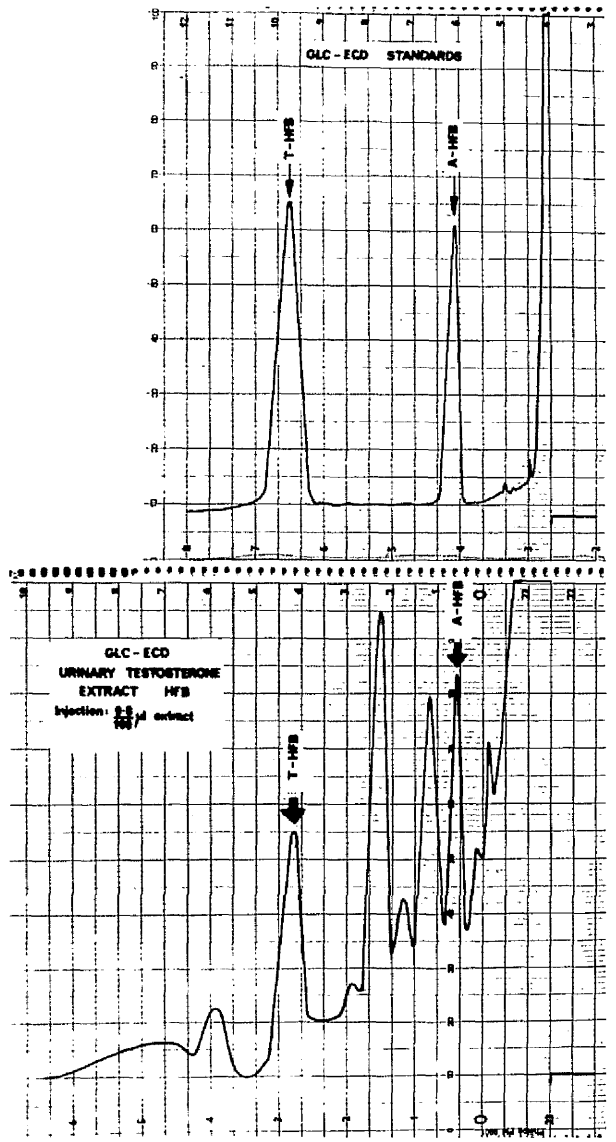


Figure 2.15 The estimation of testosterone in urine.

GLC-ECD tracing of a urine extract from a patient with bilateral undescended testes.

A. Standard trace

12.5 ng A-HFB gives a peak area of 699 mm^2.

5 ng T-HFB gives a peak area of 1,477 mm^2.

B. Urine extract

12.5 ng of the internal standard A-HFB gives a peak area of 603 mm^2. Since this peak area differs from that obtained in the standard trace (Figure 2.15A) the corresponding peak area for 5 ng T-HFB in the second trace is therefore:

$$\frac{1,477 \times 603}{699} \ mm^2$$

But the peak area of native T-HFB in the second trace is 1,020 mm^2. Therefore there are

$$\frac{699 \times 5 \times 1,020}{1,477 \times 603} \text{ ng T-HFB in the sample injected (9.8 µl of 100 µl)}$$

and $\frac{699 \times 5 \times 1,020 \times 100}{1,477 \times 603 \times 9.8}$ ng T-HFB in the urine residue.

Now $^1/_{10}$ of the residue was removed for calculation of recovery. The recovery was 27.95 per cent.*

Therefore if the recovery of the native steroid is equivalent to the recovery of ^{3}H-T added to the urine, then there were

$$\frac{699 \times 5 \times 1,020 \times 100 \times 100 \times 10}{1,477 \times 603 \times 9.8 \times 27.95 \times 9} \text{ ng of testosterone in the urine extract (100 ml).}$$

The total 24-hour urine volume was 1,540 ml.

Therefore there are $\frac{699 \times 5 \times 1,020 \times 100 \times 100 \times 10 \times 1,540}{1,477 \times 603 \times 9.8 \times 27.95 \times 9 \times 100}$ ng

of testosterone excreted per 24 hours;

ie $\frac{699 \times 5 \times 1,020 \times 1,540}{1,477 \times 603 \times 9.8 \times 27.95 \times 9}$ µg of testosterone excreted per 24 hours;

ie 2.501 µg of testosterone per 24 hours.

* Low recovery. Normal recovery range 40-60 per cent.

DETERMINATION OF URINARY ANDROGEN METABOLITES

The persistent use of 17-oxosteroid excretion assays in clinical medicine is attributed to historical and technological factors rather than endocrinological rationale. The urinary 11-deoxy-17-oxosteroids ($C_{19}O_2$ steroids) of gonadal origin are measured with identical metabolites derived from the adrenal secretion of dehydroepiandrosterone sulphate. The urinary 11-oxo-17-oxosteroids ($C_{19}O_3$ steroids) have an entirely different origin and significance (Goldzieher and Beering, 1969) being metabolites of corticosteroids and 11β-hydroxyandrostenedione, and yet these latter are included in the determination of total urinary 17-oxosteroids. The estimation is further complicated by the non-specificity of the Zimmermann colour reaction. This non-specificity has been found misleading in the study of various endocrinological states (Mahesh et al., 1964; Gleispach et al., 1969).

As the total urinary 17-oxosteroid excretion in children is low and, moreover, the urinary excretion of 17-oxosteroids in the patients selected was likely to be subnormal, a method to quantitate individual androgens and androgen metabolies in such patients must have both sensitivity and specificit A fractionation of 17-oxosteroids subsequently separately quantitated by the Zimmermann colour reaction (Kellie and Wade, 1957; Vestergaard and Claussen, 1962) was dismissed as the method had insufficient sensitivity and specificity

In the last decade many methods, which estimate the majority of urinary steroid metabolites important for differential endocrinological diagnosis, have been published. Most of these methods utilise gas-liquid chromatography as the only chromatographic separation (Haahti et al., 1961; Sparagana et al., 1963; De Paoli et al., 1963; Ruchelman and Cole, 1966; Van Kampen and Hoek, 1967; Horning et al., 1969). The method used in this investigation utilises gas-liquid chromatography as the only chromatographic step although significant initial purification is given to the urinary extract. The method

was developed for routine analyses of urinary androgen metabolites with a view to assisting in the diagnosis of patients with various gonadal problems. A flow diagram of the method is shown in Figure 2.16.

HYDROLYSIS OF URINARY CONJUGATES

The principal problem in the analysis of urinary steroids is the method of hydrolysis (Cawley et al., 1965). Most methods employ β-glucuronidase hydrolysis and subsequent acid hydrolysis of steroid sulphates[1]. Hydrolysis of both conjugates in one step by perchloric acid in anhydrous tetrahydrofuran (Jacobsohn and Lieberman, 1962; De Paoli et al., 1963) was advanced as a method of quantitative hydrolysis of 17-oxosteroids. Subsequent utilisation of this hydrolysis (Berret and McNeil, 1966; Matthijssen and Goldzieher, 1971) has revealed contaminating substances which prevent accurate quantitation of the 17-oxosteroids. Horning et al. (1969) used enzymic hydrolysis utilizing both the β-glucuronidase and sulphatase activity of their enzyme preparation.

As the β-glucuronidase powder prepared in this laboratory and used in this investigation was known to have significant sulphatase activity, the powder was used as the basis for enzymatic hydrolysis.

The hydrolysis of androgen conjugates in urine (100 ml) was effected at pH 4.6 by incubation with β-glucuronidase (750 F.u. per ml urine; 75,000 F.u.) for 72 hours at 37°C. The pH was maintained by the addition of 2 M acetate buffer (10 ml). The sulphatase activity of the enzyme was not inhibited. Following hydrolysis the urine was extracted (page 91).

Evaluation of the Hydrolysis Method

The potency of the β-glucuronidase preparation used throughout this investigation was estimated using phenolphthalein glucuronoside as substrate. The enzyme preparation was also known to have significant sulphatase activity, estimated using radioactively labelled dehydroepiandrosterone sulphate as substrate (Figure 2.12).

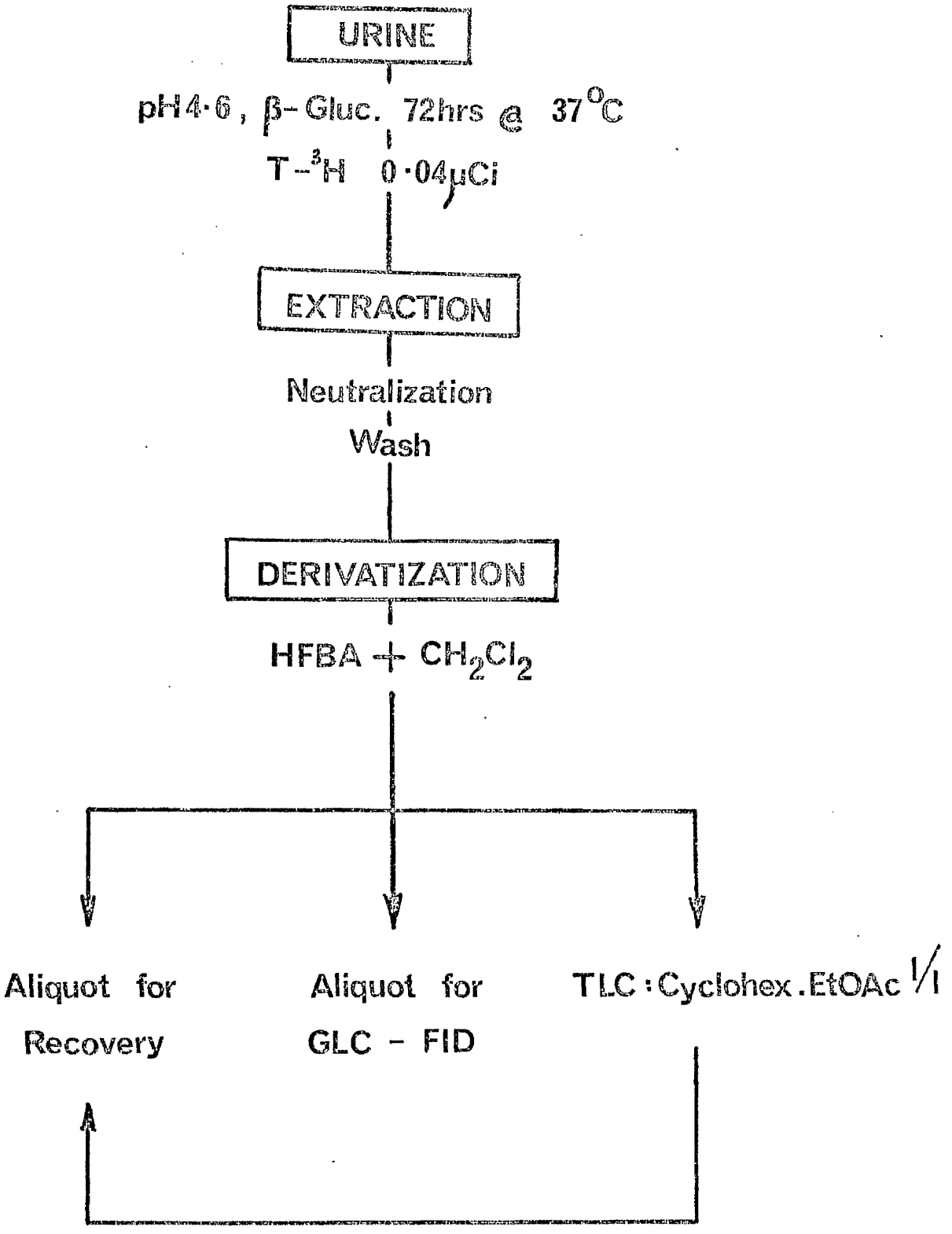


Figure 2.16 Flow diagram of procedures used in the estimation of urinary androgen metabolites.

Conjugates of epiandrosterone were used as representative of urinary androgen conjugates to assess the efficiency of the hydrolysis technique. The following experiment was undertaken. Flasks were set up in duplicate containing: epiandrosterone (30 µg) in water (50 ml); epiandrosterone-3-glucuronide (30 µg) in water (50 ml); epiandrosterone-3-sulphate (30 µg) in water (50 ml); epiandrosterone (10 µg), epiandrosterone-3-glucuronide (10 µg) and epiandrosterone-3-sulphate (10 µg) in water (50 ml); and control flasks containing water (50 ml). To all flasks were added 2 M acetate buffer (5 ml) and β-glucuronidase (750 F.u. per ml) (37,500 F.u.). The pH was adjusted to 4.6 and all flasks were incubated at 37°C for 72 hours. Free epiandrosterone in the flasks was extracted with diethyl ether (2 x 50 ml). The extracts were dried under a stream of nitrogen in a water bath at 40°C. Assay of epiandrosterone was by the Zimmermann reaction using epiandrosterone (30 µg) as the standard. The results are shown in Table 2.VI. Under the hydrolysis conditions used in this investigation there was 97 per cent hydrolysis of the glucuroside conjugates, 81 per cent hydrolysis of the sulphates and negligible destruction of free epiandrosterone. Thus this method of hydrolysis is adequate for the hydrolysis of androgen conjugates in urine.

Extraction and Preliminary Purification of Hydrolysed Urine

The hydrolysed urine was filtered through Whatman GF/A glass fibre filter into a separating flask (500 ml). [1,2,6,7-^{3}H]Testosterone (S.A. 87 Ci/mM, 36 nCi) was added to the filtrate for recovery purposes. The filtrate was extracted with diethyl ether (4 x 100 ml). The extract was reduced to small volume under nitrogen in a water bath at 40°C and washed back into the empty extraction flask with diethyl ether (100 ml). The diethyl ether was washed with 5 M sodium hydroxide (10 ml) and deionised water (20 ml) and water washing repeated until the washings were neutral to pH indicator paper. The extract was dried under nitrogen in a water bath at 40°C

TABLE 2.VI RECOVERY OF EPIANDROSTERONE FROM VARYING AMOUNTS OF EPIANDROSTERONE (EpiA), EPIANDROSTERONE GLUCURONOSIDE (EpiA-G) AND EPIANDROSTERONE SULPHATE (EpiA-S) ADDED TO WATER (50 ml) AND INCUBATED WITH β-GLUCURONIDASE (750 F.u. per ml) FOR 72 HOURS AT 37°C. ASSAY WAS BY THE ZIMMERMANN REACTION.

Test	Optical Density @ Wavelength (nm) 435	515	595	AC[1]	AC-RB	Percentage Recovery of EpiA
Reagent Blank	023	028	013	010	000	
	020	024	008	010		
Standard EpiA (30 µg)	214	485	219	268	262	
	218	486	202	276		
Incubated EpiA (30 µg)	217	473	208	260	256	97.7
	217	488	215	272		
Incubated EpiA-G (30 µg)	219	485	199	276	254	96.9
	228	473	214	252		
Incubated EpiA-S (30 µg)	202	418	196	219	212	80.9
	200	420	189	225		
Incubated EpiA + EpiA-G + EpiA-S (10 µg each)	219	452	206	239	234	89.3
	232	470	209	249		

AC = Allen correction (Allen, 1950)

RB = Reagent Blank

The recovery of ^{3}H-testosterone added initially to the urine samples indicated that recovery of free androgens (testosterone) after extraction was 99.4 per cent.

The dried extract was redissolved in 70 per cent (v/v) methanol in water (10 ml) and partitioned against hexane (10 ml) to remove lipids and other non-steroidal material (Oertel, 1961). The hexane layer was discarded and the aqueous methanol dried under nitrogen in a water bath at 40°C.

ESTERIFICATION OF STEROIDS

Derivatives of androgens have been used to facilitate electron capture detection, to exaggerate molecular shape of epimers and to decrease retention times on selective phases. The major 17-oxosteroids have been separated by gas-liquid chromatography as the free compounds (Haahti et al., 1961; De Paoli et al., 1963; Van Kampen and Hoek, 1967), as trimethylsilyl ethers (Van den Heuvel et al., 1962; Hartman and Wotiz, 1963; Horning et al., 1969), as ethylene-thioketal derivatives (Zmigrod and Lindner, 1966) and as chloromethyldimethylsilyl ethers (Matthijssen and Goldzieher, 1971).

In this investigation the heptafluorobutyrate derivatives of androgens and androgen metabolites were prepared. Conditions of derivatization were those developed for plasma and urinary testosterone derivatization.

Esterification of Urinary Extracts

Dried residues were placed in a vacuum desiccator for one hour. The residues were dissolved in dichloromethane (2 ml) and esterified with heptafluorobutyric anhydride (100 µl) at room temperature for 30 minutes. Excess reagent was removed by evaporation to dryness at room temperature under nitrogen. These reaction conditions favour esterification at hydroxyl groups but prevent enol formation (and thus esterification of Δ^{4}-3-oxosteroids)

Dried esters were then dissolved in hexane (2 ml) and partitioned against 70 per cent (v/v) methanol in water to remove unesterified steroids. A recovery of 87 per cent radioactivity from the hexane fraction at this stage indicated a near total esterification of testosterone.

Preparation of Standard Androgen Heptafluorobutyrates

A standard solution containing 5α-androstane-3α,17β-diol (1 mg), 5β-androstane-3α,17β-diol (1 mg), Δ^5-androstene-3β,17β-diol (1 mg), 5α-androstane-3β,17β-diol (1 mg), androsterone (1 mg), aetiocholanolone (1 mg), dehydroepiandrosterone (1 mg), epiandrosterone (1 mg), 11β-hydroxyandrosterone (1 mg), 11β-hydroxyaetiocholanolone (1 mg) and radioactively labelled [1α,2α-^{3}H]androsterone (S.A. 40 Ci/mM, 20 nCi) was prepared in dichloromethane (5 ml). The steroids in this solution were esterified with heptafluorobutyric anhydride (100 µl) at room temperature for 30 minutes. Excess reagent was removed by evaporation to dryness at room temperature under a stream of nitrogen. The dried esterified steroids were dissolved in ethyl acetate (5 ml). An aliquot (100 µl) was applied to a silica thin layer and, after the application of standard androsterone and androsterone-3-heptafluorobutyrate, the TLC plate was developed in the solvent system cyclohexane:ethyl acetate (1:1, v/v). The standard steroids were located under UV light (356 nm) after spraying with Rhodamine 6G (0.1 per cent, w/v, in ethanol). The TLC plate was radiochromatographically scanned and the areas of radioactively labelled androsterone and androsterone-3-heptafluorobutyrate were scraped from the plate and transferred to a counting vial. The percentage esterification was calculated from the results of liquid scintillation counting of these samples. A conversion of 92 per cent androsterone to androsterone-3-heptafluorobutyrate (as representative of the standard androgens) is achieved by this method.

GAS LIQUID CHROMATOGRAPHY

Liquid phase and operating conditions for the GLC method were required by which separation of the steroid esters present in the sample could be effected. After several liquid phases had been tested, the highly polar polyester, neopentyl glycol sebacate (N.G.Seb.) was chosen as this gave baseline separation of the steroid heptafluorobutyrates studied. Hartman and Wotiz (1963) described this liquid phase for separation of

trimethylsilyl ether derivatives of androgen and progesterone metabolites.

A five-foot column with 3 per cent coating of temperature stabilized N.G.Seb. on Gas Chrom Q, 100-120 mesh, was prepared by the filtration method in vacuo previously described. The column was conditioned for 24 hours at 220°C with a carrier gas flow rate of 60 ml per minute.

The following parameters were regulated for operation of FID. The column temperature was operated isothermally at 190°C, and the injection port heater maintained at 220°C. The gas flow rates used with FID were: oxygen-free nitrogen (carrier gas) - 40 ml per minute; hydrogen - 40 ml per minute; air - 650 ml per minute.

Prior to the analysis of each group of samples, a known amount of the HFB-esters of standard steroids was injected into the gas chromatograph using a 10 µl S.G.E. micro-syringe. The average retention time for the last compound 11β-hydroxyaetiocholanolone was 150 minutes and for the internal standard (5α-dihydrotestosterone-17-heptafluorobutyrate) 45 minutes (Figure 2.17). For the test samples 5-10 µl of the derivatized sample extract was used. The peaks were identified by comparing their relative retention times with those of the standard steroids (Figure 2.18).

Linearity of the Detector

Linearity of the detector response was determined using peak areas calculated from the product of the peak height and the width of the peak at half peak height. The internal standard, 5α-dihydrotestosterone-17-HFB, was injected with each sample, Although the response for the heptafluorobutyrates of 5α-androstane-3α,17β-diol, androsterone and 11β-hydroxyandrosterone (as representative of the standard steroid esters) and also the internal standard, 5α-dihydrotestosterone-17-HFB, was proportional to the amount injected (Figure 2.19), the response of individual steroid esters was slightly less so. Thus the relative response (the ratio of the peak area of each standard steroid HFB to the peak area of the same amount

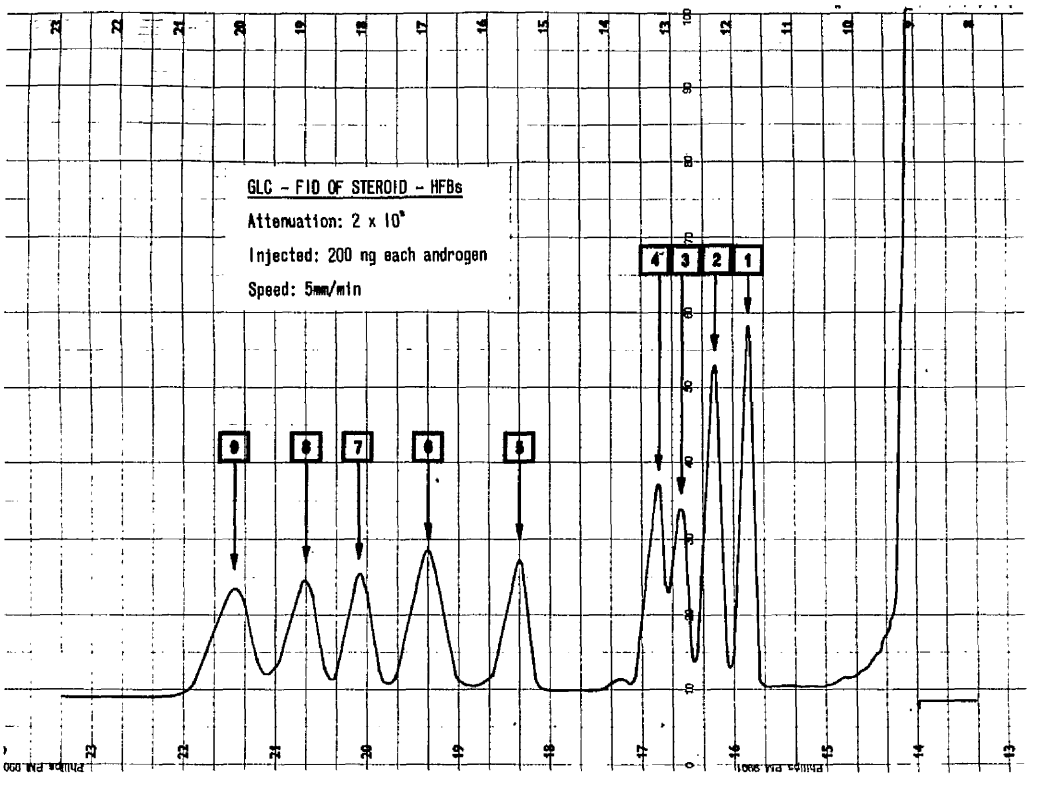


Figure 2.17 Gas liquid chromatographic analysis with flame ionization detection of a standard mixture of esterified androgen metabolites and the internal standard, 5α-dihydrotestosterone-HFB (compound 9).

KEY:
1. 5α-Androstane-3α,17β-diol-HFB
2. 5β-Androstane-3α,17β-diol-HFB
3. Δ^5-Androstene-3β,17β-diol-HFB
4. 5α-Androstane-3β,17β-diol-HFB
5. Androsterone-HFB
6. Aetiocholanolone-HFB
7. Dehydroepiandrosterone-HFB
8. Epiandrosterone-HFB
9. 5α-Dihydrotestosterone-HFB (internal standard).

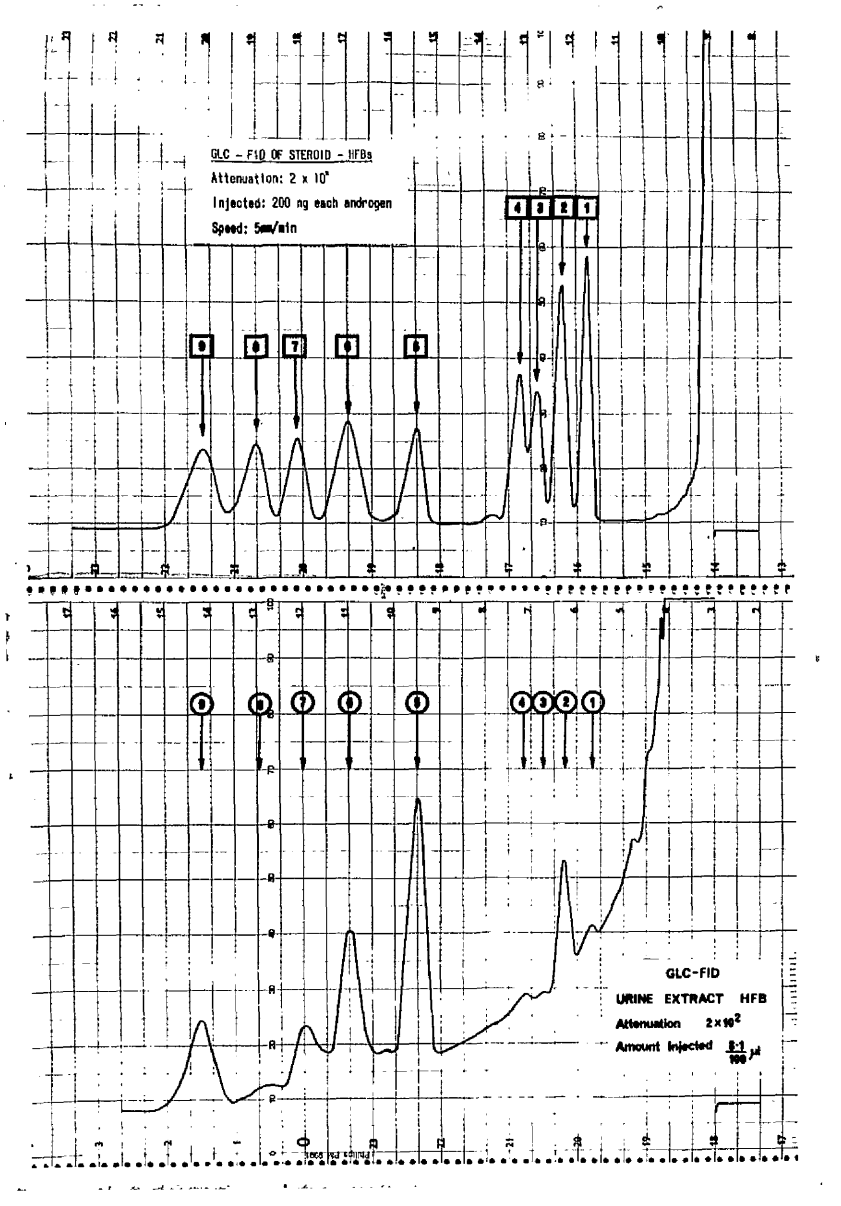


Figure 2.18 Gas liquid chromatographic analysis with flame ionization detection of an esterified standard mixture of androgen metabolites (A) and a derivatized urine extract (B). Peaks were identified by comparison of their relative retention times with those of esterified standards.

Peaks 1 to 9 were identified on the previous page (Figure 2.17).

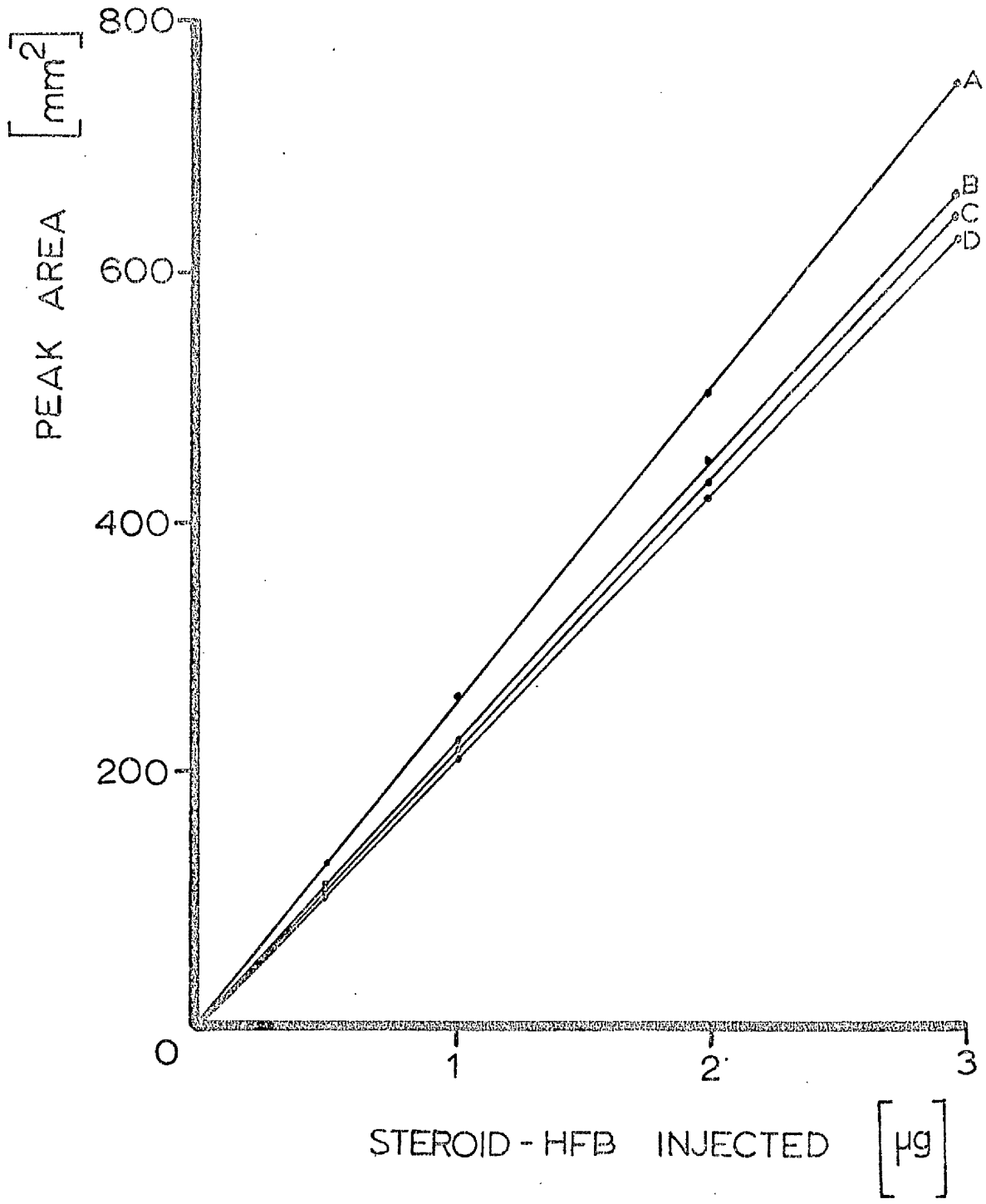


Figure 2.19 Linearity of the flame ionization detector to varying concentrations of 5α-diol (A), androsterone (B), 11β-hydroxyandrosterone (C) and 5α-dihydrotestosterone (D) determined from peak area measurements.

of internal standard) was used in the quantitation of androgen metabolites. The relative response for each steroid ester was calculated from 50 GLC tracings of the mixture of HFB derivatives of standard steroids used at various times throughout this investigation (Table 2.VII). The coefficient of variation for different steroid esters ranged between 4.5 per cent and 9 per cent indicating satisfactory reproducibility of the method.

Quantitation of Urinary Androgen Metabolites

Prior to the analysis of each group of samples, a known amount of HFB-esters of standard steroids was injected into the gas chromatograph. A known amount of 5α-dihydrotestosterone-17-HFB was injected as an internal standard together with the esterified urinary extract. Quantitation was made by comparison of the peak area of steroid HFB in the sample to the peak area of the internal standard injected with the sample, with the inverse of the relative response (ie peak area of internal standard injected with standard to peak area of standard steroid HFB). Correction was made for the aliquot injected, and for the percentage recovery as estimated by the recovery of ^{3}H-testosterone added initially to the samples. The results were corrected to the total 24-hour urine excretion. An example of the estimation of androgen metabolites in a urine sample is given in Figure 2.20 and the accompanying explanatory text.

TABLE 2.VII THE RELATIVE RESPONSE OF THE HEPTAFLUOROBUTYRATE ESTERS OF STANDARD STEROIDS (50 SEPARATE GLC-FID ANALYSES)

Steroid	Relative Response*		
	Mean	SD	Coefficient of Variation
5α-Androstane-3α,17β-diol	1.154	0.070	6.061
5β-Androstane-3α,17β-diol	1.115	0.058	5.202
Δ^5-Androstene-3β,17β-diol	0.968	0.067	6.920
5α-Androstane-3β,17β-diol	1.385	0.098	7.074
Androsterone	1.013	0.075	7.404
Aetiocholanolone	1.074	0.047	4.376
Dehydroepiandrosterone	0.869	0.051	5.869
Epiandrosterone	1.117	0.063	5.640
11β-Hydroxyandrosterone	1.003	0.046	4.586
11β-Hydroxyaetiocholanolone	0.621	0.053	8.535

* Relative to the internal standard 5α-dihydrotestosterone-17-HFB

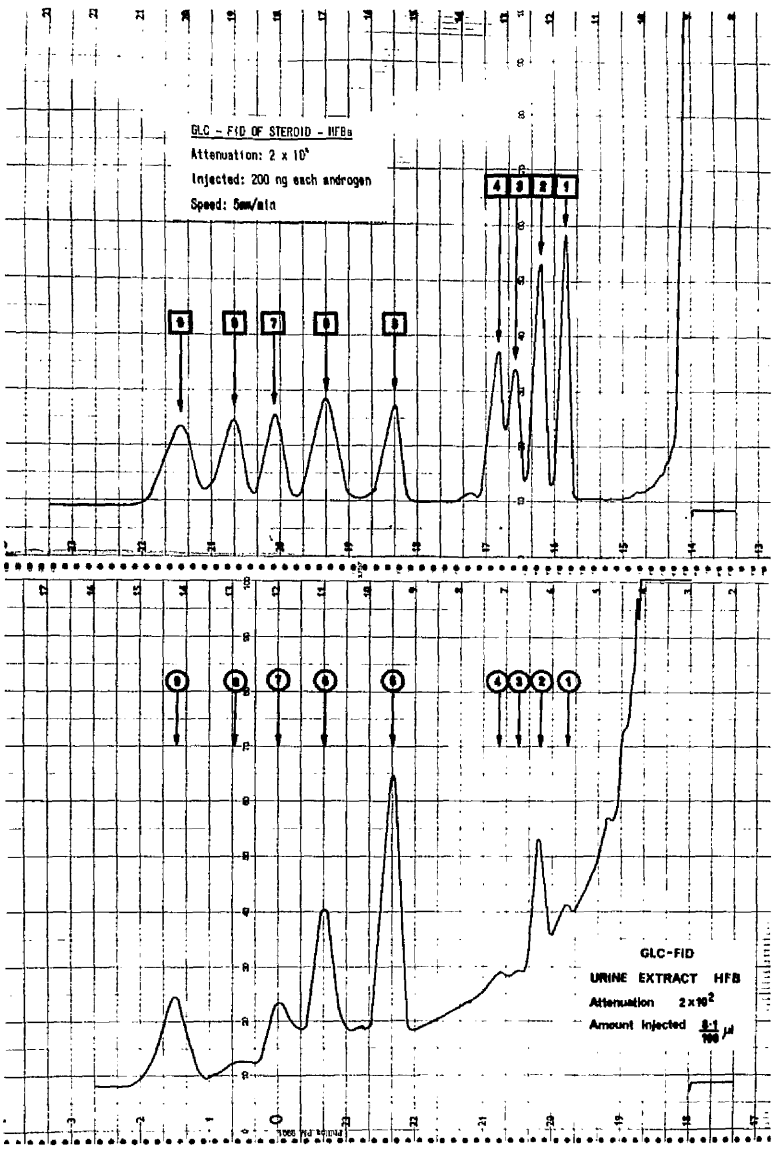


Figure 2.20 The calculation of urinary excretion of androgen metabolites. GLC-FID tracing of a urine extract from a patient with delayed puberty.

A. Standard trace

200 ng of 5α-DHT-HFB gives a peak area of 504 mm^2. The relative response (ratio of the peak area of standard steroid to the peak area of the internal standard) for each standard steroid ester was:

5α-Androstane-3α,17β-diol	1.198
5β-Androstane-3α,17β-diol	1.187
Δ^5-Androstene-3β,17β-diol	0.905
5α-Androstane-3β,17β-diol	1.037
Androsterone	0.902
Aetiocholanolone	1.181
Dehydroepiandrosterone	0.806
Epiandrosterone	0.984

B. Urine extract

200 ng of 5α-DHT-HFB gives a peak area of 488 mm^2. Since this peak area differs from that obtained in the standard trace (Figure 2.20A) the corresponding peak area for 200 ng of steroid ester in the second trace is therefore:

$$488 \times \text{Relative response (RR)}$$

But the peak areas of native androgen metabolites in the second trace (AREA) are:

5α-Androstane-3α,17β-diol	40 mm^2
5β-Androstane-3α,17β-diol	312 mm^2
Δ^5-Androstene-3β,17β-diol	8 mm^2
5α-Androstane-3β,17β-diol	45 mm^2
Androsterone	1,062 mm^2
Aetiocholanolone	650 mm^2
Dehydroepiandrosterone	351 mm^2
Epiandrosterone	60 mm^2

Therefore there are $\frac{200 \times \text{AREA}}{488 \times \text{RR}}$ ng of steroid-HFB in the sample injected (8.1 µl of 100 µl),

and $\frac{200 \times \text{AREA} \times 100}{488 \times \text{RR} \times 8.1}$ ng of steroid-HFB in the urine residue.

But $^3/_{10}$ of the residue were removed previously for calculation of recovery and percentage esterified. The recovery was 87.49 per cent and 88.4 per cent was esterified. Therefore if the recovery of the native androgen metabolites is equivalent to the recovery of ^{3}H-T added initially to the urine, then there were

$$\frac{200 \times AREA \times 100 \times 100 \times 10 \times 100}{488 \times RR \times 8.1 \times 87.49 \times 7 \times 88.4}$$ ng of steroid in the urine extract (100 ml)

The total 24-hour urine volume was 1,280 ml

Therefore there are

$$\frac{200 \times AREA \times 100 \times 100 \times 10 \times 1,280}{488 \times RR \times 8.1 \times 87.49 \times 7 \times 88.4}$$ ng of steroid excreted per 24 hours.

ie $\frac{AREA \times 0.1196}{RR}$ µg of steroid excreted per 24 hours.

Substitution then gives the following urinary excretions:

	µg per 24 hours
5α-Androstane-3α,17β-diol	4
5β-Androstane-3α,17β-diol	31
Δ^5-Androstene-3β,17β-diol	1
5α-Androstane-3β,17β-diol	5
Androsterone	141
Aetiocholanolone	66
Dehydroepiandrosterone	52
Epiandrosterone	7.

CLINICAL PROTOCOL FOR TESTICULAR FUNCTION TESTS

It will be seen, in a subsequent chapter of this thesis (Chapter 3), that there are four results given for most urinary analyses and two results given for plasma analyses. At the clinical level these patients were given Human Chorionic Gonadotrophin (HCG) (6,000 I.U. intramuscularly at 6 am daily) for three days after the collection of a basal 24-hour urine sample and peripheral venous blood (20 ml). Throughout the stimulation period 24-hour urine collections were made and a second sample of peripheral venous blood (20 ml) was taken on the third day of stimulation (ie the fourth day of test).

Blood was withdrawn between 11 am and noon, on the days of collection, into heparinized tubes, and the plasma was separated immediately by centrifugation. The plasma was either processed immediately or stored at $-15^{\circ}C$ until assayed. Urine specimens were collected in screw top bottles to which chloroform (5 ml) was added as a preservative. All collections were made under the supervision of the nursing staff.

Other forms of patient investigation (growth hormone response to insulin hypoglycemia, thyroid function tests and tests of the hypothalamic-pituitary axis) were carried out, when required, on the day preceding basal urine collection. The information derived from these tests has been used to support the thesis that by studying the nature of urinary testosterone metabolites in conjunction with the testicular ability to elaborate testosterone, an assessment of tissue sensitivity to (and probably dependence on) testosterone can be made.

The typical clinical protocol is given in Table 2.VIII.

TABLE 2.VIII CLINICAL PROTOCOL USED FOR TESTICULAR FUNCTION TESTS

Day	Test Day	Clinical Protocol
A		Admission. Routine clinical tests.
B	Pretest	Other investigations, as required.
C	Basal	24-hour urine collection. 20 ml peripheral venous blood at 11 am.
D	Day 1	24-hour urine collection. 6,000 I.U. HCG IM at 6 am.
E	Day 2	24-hour urine collection. 6,000 I.U. HCG IM at 6 am.
F	Day 3	24-hour urine collection. 6,000 I.U. HCG IM at 6 am. 20 ml peripheral venous blood at 11 am.
G		Discharge home.

CHAPTER 3

R E S U L T S

INTRODUCTION

In this work, plasma testosterone, urinary testosterone and most of the urinary testosterone metabolites have been assayed in the basal state and over a three-day course of human chorionic gonadotrophin (HCG; 6,000 I.U. intramuscularly daily at 6 am). These metabolites included the total 17-oxosteroids, androsterone, aetiocholanolone, dehydroepiandrosterone (DHA), epiandrosterone, 5α-androstanediol (5α-diol), 5β-androstanediol (5β-diol), Δ^5-androstenediol (Δ^5-diol) and 5α-androstane-3β,17β-diol (3β-diol).

Additionally, for each compound a stimulation index was calculated by dividing the excretion on the third day of HCG stimulation by the basal level. Clearly values of this stimulation index above unity indicate increased excretion (stimulation) whilst values less than unity indicate decreased excretion (no stimulation) following HCG administration.

Since I was unable, for ethical reasons, to undertake a similar study on normal boys, I have utilized data reported in the literature for comparison of the results from this series of 'abnormal' boys.

PATIENTS UNDER STUDY

The method for evaluating testicular function, as outlined in the previous chapter, has been used to investigate several patients of abnormal karyotype and some patients with various genital abnormalities. Of the 76 cases presented, testicular function was assessed in 75 by their response to intramuscularly administered HCG. All urine collections, with the exception of those from one adult patient, were made in hospital under the supervision of the nursing staff.

The patients and their clinical conditions are tabulated in Table 3.I. Their ages ranged from 5 months to 26 years, although the majority were in the paediatric age range. The actual protocols for the patients are

TABLE 3.I CLINICAL CONDITION FOR WHICH INVESTIGATION UNDERTAKEN

Genotype or phenotype	No. of patients investigated
Testicular feminization syndrome (46,XY female)	4
Klinefelter's syndrome (47,XXY)	1
Klinefelter's syndrome (46,XX)	1*
XO/XY syndrome (45,XO/46,XY)	2
XX/XY syndrome (46,XX/46,XY)	1
Short stature *per se*	18
Short stature with hypogonadism (microgenitalia)	11**
Hypogonadism (microgenitalia clinically)	6
Hypogonadism with bilateral undescended testes	9
Hypogonadism with unilateral undescended testis	4
Delayed puberty	3
Cushing's syndrome	4
Prader-Willi syndrome	3
Male Turner's syndrome	2
Laurence-Moon-Biedl syndrome	1
? Anorchia (clinically undescended testes)	3
Precocious puberty	2
? Infertility	1
Post cyclophosphamide therapy (nephrotic syndrome)	1

* 1 patient re-investigated following therapy

** 2 patients re-investigated following therapy

collected in a later section of this thesis (Chapter 5). Patient information, diagnosis and individual results of investigations are reported there. Patients in the text are thus identified by a protocol number.

URINARY CREATININE RESULTS

Considerable fluctuation was found in the volumes of serial 24-hour urine specimens from individual patients despite the well-organised ward conditions for urine collection. The completeness of many specimens, particularly those from younger children, was therefore questioned. The daily urinary creatinine excretion was estimated on all 24-hour urine collections and that value used as a measure of the completeness of each collection. These results are shown in Table 3.II.

From column E (Table 3.II), where the standard deviation (SD) for each group of urine collections is shown, it is clear that there was considerable disparity in the daily urine volumes for many individuals. However, from analyses of the coefficient of variation (columns G and H, Table 3.II), it is clear that the urinary excretion of creatinine is a much more precise estimate of urinary volume completeness. Calculation of the mean values of columns G and H gave the mean coefficient of variation of urine volume and the mean coefficient of variation of daily creatinine excretion respectively. The mean coefficient of variation of urine volume was 29.52 per cent (SD = 11.66 per cent) and the mean value of the coefficient of variation of creatinine excretion was 16.84 (SD = 9.71 per cent). This difference is statistically highly significant ($p < 0.0001$), justifying the use of the daily urinary excretion of creatinine as an index of the completeness of 24-hour urine collection in these patients.

When the patients investigated were grouped according to age, height and pubertal status, and the mean daily creatinine excretion (column D, Table 3.II) for each group plotted against the mean of the age group, an interesting correlation was found. This is shown in the graph, Figure 3.1.

TABLE 3.II COMPARISON OF THE DAILY URINARY EXCRETION OF CREATININE WITH THE DAILY URINE VOLUME

Protocol	Age (Years)	Mean 24-hour Excretion		Standard Deviation		Coefficient of Variation	
		Urine ml	Creatinine mg	Urine ml	Creatinine mg	Urine %	Creatinine %
A	B	C	D	E	F	G	H
1	0.4	385	61	136	10	35.32	16.39
2	2.8	367	149	127	31	34.60	20.81
3	3.3	513	246	120	33	23.39	13.41
4	10.1	1,436	586	479	53	33.36	9.04
5	6.0	249	299	83	87	33.18	29.10
6	12.3	920	567	331	146	35.98	25.77
7	14.8	915	821	206	110	22.51	13.40
8	8.7	585	587	254	49	43.42	8.35
9	14.0	925	1,190	309	113	33.41	9.50
10	8.1	311	241	156	73	50.16	30.29
11	8.5	377	279	120	54	31.83	19.35
12	10.1	721	133	427	15	59.22	11.28
13	11.0	1,472	385	368	79	25.00	20.52
14	11.5	565	338	173	61	30.62	18.05
15	12.2	700	664	230	104	32.86	15.66
16	14.3	879	579	279	126	31.74	21.76
17	15.0	1,276	785	310	56	24.29	7.13
18	5.4	440	109	240	27	54.54	24.77
19	6.0	710	147	320	48	45.07	32.65
20	8.3	407	456	122	45	29.98	9.87

21	10.1	752	354	335	86	44.55	24.29
22	10.3	510	616	109	99	21.37	16.15
24	15.0	1,163	619	120	24	10.32	3.88
25	15.3	1,105	740	475	86	42.99	11.62
26	4.1	266	168	49	20	18.42	11.90
27	6.0	339	215	117	30	34.51	13.95
28	7.3	509	276	148	36	29.08	13.04
29	8.2	542	203	215	41	39.67	20.20
30	10.8	770	663	367	303	47.66	45.70
31	10.8	735	537	110	54	14.97	10.06
32	11.0	766	613	73	59	9.53	9.62
33	11.2	466	265	210	115	45.06	43.40
34	11.9	246	290	69	31	28.05	10.69
35	13.5	793	594	328	128	41.36	21.55
36	14.8	491	669	45	50	9.16	7.47
37	15.0	786	1,260	226	133	28.75	10.56
38	16.1	769	518	147	62	19.12	11.97
39	19.0	1,132	683	392	54	34.63	7.91
40	4.1	335	106	103	8	30.75	7.55
41	4.2	50	33	22	14	44.00	42.42
42	13.0	1,099	446	340	56	30.94	12.56
43	14.0	1,076	544	214	73	19.89	13.42
44	14.0	923	770	123	40	13.33	5.19
45	16.0	886	788	177	156	19.98	19.80

TABLE 3.II (continued)

Protocol	Age (Years)	Mean 24-hour Excretion		Standard Deviation		Coefficient of Variation	
		Urine ml	Creatinine mg	Urine ml	Creatinine mg	Urine %	Creatinine %
A	B	C	D	E	F	G	H
46	16.0	1,526	652	447	75	29.29	11.50
47	16.0	1,074	599	223	94	20.76	15.69
48	16.0	1,588	828	251	106	15.81	12.80
49	16.1	503	650	173	118	34.39	18.15
50	17.1	825	1,020	270	316	32.73	30.98
51	3.8	815	320	165	36	20.25	11.25
52	6.8	550	80	171	22	31.09	27.50
53	10.9	1,266	648	380	14	30.02	2.16
54	11.0	2,198	525	890	179	40.49	34.10
55	12.6	1,655	683	746	142	45.08	20.79
56	13.7	1,429	672	349	31	24.42	4.61
57	13.9	552	472	117	96	21.20	20.34
58	4.5	761	480	253	29	33.25	6.04
59	5.4	528	272	69	20	13.07	7.35
60	9.5	724	498	291	149	40.19	29.92
61	9.6	313	433	64	78	20.45	18.01
62	10.0	300	221	58	5	19.33	2.26
63	10.9	1,355	541	173	64	12.77	11.83
64	14.4	474	452	127	93	26.79	20.58

65	15.0	2,385	782	214	65	8.97	8.31
66	15.6	1,098	611	656	202	59.74	33.06
67	20.0	789	654	127	72	16.10	11.01
68	26.0	1,162	1,380	186	159	16.01	11.52
69	12.1	1,223	458	516	137	42.19	29.91
70	19.9	1,328	650	208	32	15.66	4.92
71	6.3	352	125	95	33	26.99	26.40
72	8.0	523	394	130	101	24.86	25.63
73	15.5	362	568	76	49	20.99	8.63
74	8.3	914	246	232	55	25.38	22.36
75	7.0	914	426	122	54	13.35	12.68
76	9.0	576	625	173	121	30.03	19.36
77	9.5	789	820	203	80	25.73	9.76
78	5.0	579	507	233	50	40.24	9.86
79	17.2	825	1,020	270	304	32.73	29.80
80	14.0	925	1,191	309	113	33.41	9.49
						29.52	16.84

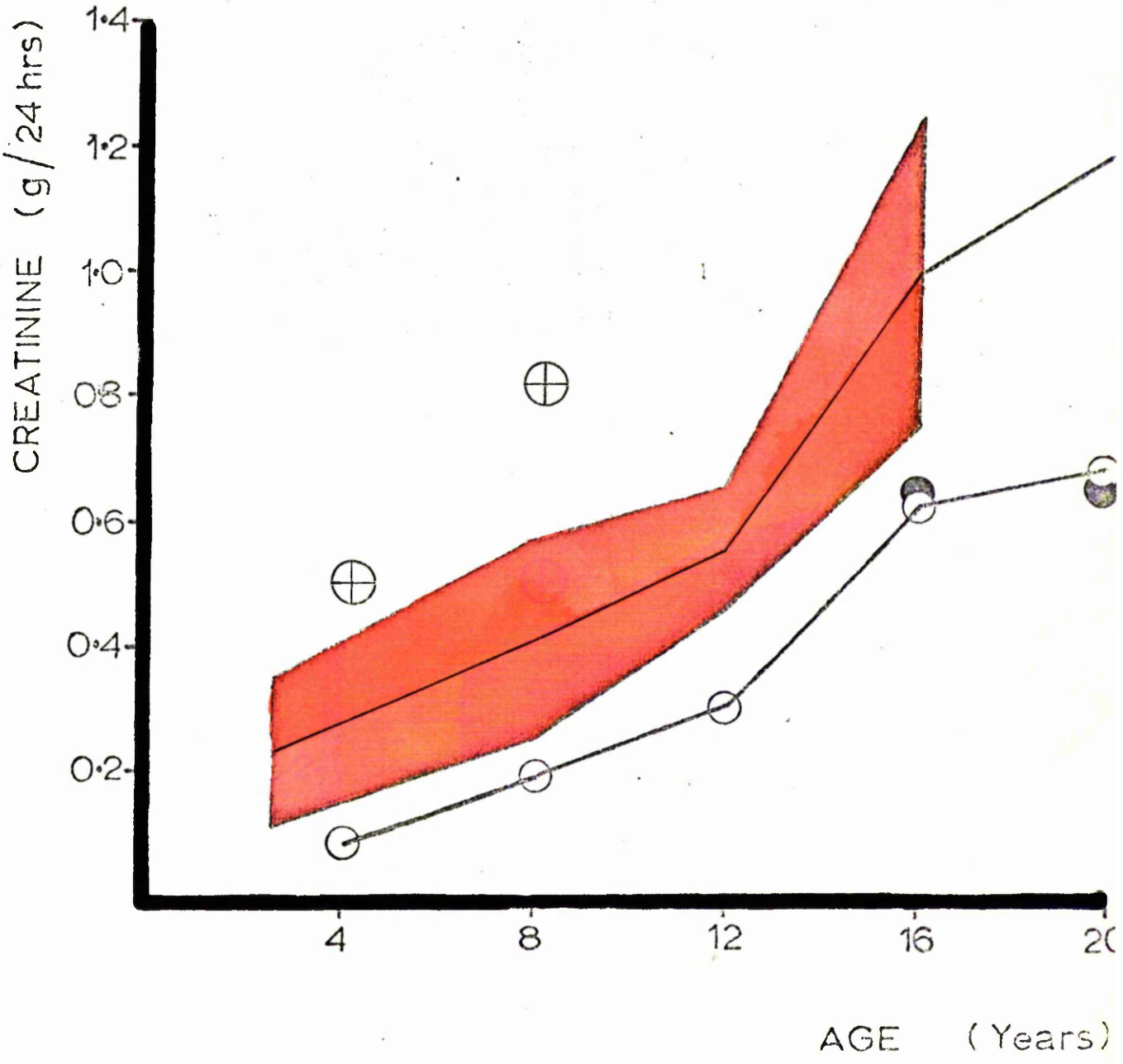


Figure 3.1 The mean daily urinary creatinine excretion of patients grouped by age, height and pubertal status. The shaded area represents the mean $\pm$ 1 SD of daily urinary creatinine excretion of patients with normal stature (height percentiles 10-90).

KEY:
- Patients with delayed puberty
- Patients with small stature (height percentiles <3)
- Patients with precocious puberty (height percentiles >97

The shaded area of the graph represents the range ($\pm$ 1 SD) of daily creatinine excretion for patients in the series of normal or near normal stature (height percentiles 10-90). This normal range of daily creatinine excretion is slightly lower, but not significantly different from those found by other workers (Clark et al., 1951; Applegarth et al., 1968). Note that the normal daily creatinine excretion rises throughout childhood and rapidly increases in parallel with the normal growth spurt at puberty. This rapid increase probably reflects the increasing muscle mass during adolescence.

The lower curve (Figure 3.1) represents the mean daily creatinine excretion in patients of short stature (height percentile <3). The daily excretion of creatinine by such patients is significantly below normal throughout all the age groups studied ($0.0005 < p \leq 0.05$). It is of interest and probably of clinical significance that the creatinine excretion in these patients parallels, at a lower level, that of normal patients.

Also in Figure 3.1, the mean daily creatinine excretion in a group of patients with delayed puberty is also lower than normal. The creatinine excretion in these patients increases at the prepubertal rate and approaches the creatinine excretion found in older patients with short stature alone. The creatinine excretion in these patients was significantly below normal ($0.01 < p < 0.05$).

The two points above the normal range represent the creatinine excretion in two patients with precocious puberty. Individual creatinine excretions were significantly higher in these patients ($0.002 < p < 0.02$) than normal. The creatinine excretion by these patients was more related to bone age. The higher creatinine excretion in these patients is probably a combination of the early growth spurt and the increased androgens of adrenal and gonadal origin.

As expected from the above results, the urinary excretion of creatinine was significantly correlated with pubertal status ($r = 0.680$), age ($r = 0.774$) and bone age ($r = 0.832$).

URINARY 17-OXOSTEROID RESULTS

The excretion of 17-oxosteroids was routinely estimated on all 24-hour urine collections to give an estimate of the amount of steroidal material present. The 17-oxosteroid response to HCG administered intramuscularly is shown in Table 3.III.

The basal excretion of 17-oxosteroids (column C, Table 3.III) is low in the majority of the patients investigated. This was particularly noted in patients in the adolescent age group. These values were analysed in relation to age, height and pubertal status. Owing to the small number of 'near-normal' subjects investigated, normal values published by Prout and Snaith (1958) were used for comparison. It has been previously shown (Gray, 1973) that the values reported by these authors are not significantly different from normal values obtained in this laboratory (Royal Hospital for Sick Children, Yorkhill, Glasgow). The method nearest to that used in this investigation was used for comparison (ie method 4 of Prout and Snaith using spectrophotometric readings of the Zimmermann colour at three wavelengths and the application of the Allen correction).

The patients investigated were grouped according to age, height and pubertal status. In Figure 3.2 the mean basal 17-oxosteroid excretion for each group was plotted against the mean of the age group. The shaded area of the graph represents the range ($\pm$ 1 SD) of daily 17-oxosteroid excretion in the normal patients studied by Prout and Snaith (1958). The basal 17-oxosteroid excretion of all 'near-normal' patients in this investigation were within that range.

The basal urinary excretion of 17-oxosteroids in early prepubertal patients of short stature (height percentile <3) was not significantly different from the normal values of Prout and Snaith (1958). However, in older patients of short stature (8-11 years) the basal 17-oxosteroid excretion was just significantly below normal ($0.02 < p < 0.05$) whilst

TABLE 3.III THE DAILY URINARY EXCRETION OF 17-OXOSTEROIDS AND RESPONSE TO ADMINISTERED HCG

Protocol	Age (Years)	Urinary 17-oxosteroids (mg/24 hrs)				Stimulation Index $\frac{\text{Day 3}}{\text{Basal}}$
		Basal	Day 1	Day 2	Day 3	
A	B	C	D	E	F	G
1	0.4	0.20	0.27	0.21	0.12	0.60
2	2.8	0.12	0.12	0.23	0.31	2.58
3	3.3	0.58	0.15	0.03	0.03	0.05
4	10.1	0.58	0.58	1.18	1.35	2.33
5	6.0	0.04	0.24	0.53	0.32	8.00
6	12.3	2.10	3.08	1.33	0.83	0.40
7	14.8	0.02	0.88	0.85	-	-
8	8.7	1.02	0.79	1.01	1.03	1.01
9	14.0	1.42	2.12	1.24	2.07	1.46
10	8.1	0.10	0.04	0.04	0.02	0.20
11	8.5	0.06	0.16	0.82	1.01	16.83
12	10.1	0.27	0.36	0.58	0.70	2.59
13	11.0	0.37	1.10	0.88	0.42	1.14
14	11.5	0.45	0.41	0.37	0.44	0.98
15	12.2	0.99	0.62	0.09	0.30	0.30
16	14.3	0.99	1.02	0.92	0.78	0.79
17	15.0	0.43	0.75	1.18	1.47	3.42
18	5.4	0.19	0.10	0.11	0.11	0.58
19	6.0	0.38	ND*	ND	0.14	0.37
20	8.3	0.38	0.70	0.63	0.64	1.68

21	10.1	1.71	4.15	3.39	2.56	1.50
22	10.3	1.02	0.93	1.01	0.64	0.63
24	15.0	1.43	1.21	1.69	2.37	1.66
25	15.3	1.44	0.59	0.56	-	-
26	4.1	0.16	0.10	0.18	0.16	1.00
27	6.0	0.11	0.11	0.02	0.11	1.00
28	7.3	0.23	0.34	0.15	0.09	0.39
29	8.2	0.35	0.28	0.37	0.35	1.00
30	10.8	1.49	1.38	1.51	1.61	1.08
31	10.8	0.40	0.65	0.89	0.97	2.43
32	11.0	0.66	0.71	0.33	0.09	0.14
33	11.2	0.19	0.20	0.21	0.29	1.53
34	11.9	1.02	0.64	0.48	0.62	0.61
35	13.5	0.83	0.86	1.34	1.29	1.55
36	14.8	0.20	0.35	0.15	0.30	1.50
37	15.0	0.72	1.28	1.24	1.16	1.61
38	16.1	0.95	1.28	1.71	0.58	0.61
39	19.0	0.25	0.21	0.60	0.48	1.92
40	4.1	0.13	0.14	0.18	0.19	1.46
41	4.2	0.15	0.11	0.08	0.05	0.33
42	13.0	0.30	0.31	0.55	0.61	2.03
43	14.0	0.65	1.40	0.72	0.57	0.88
44	14.0	2.45	2.90	3.35	3.99	1.63
45	16.0	1.38	1.33	1.57	1.08	0.78

TABLE 3.III (continued)

Protocol	Age (Years)	Urinary 17-oxosteroids (mg/24 hrs)				Stimulation Index $\frac{\text{Day 3}}{\text{Basal}}$
		Basal	Day 1	Day 2	Day 3	
A	B	C	D	E	F	G
46	16.0	1.14	1.78	1.55	2.26	1.98
47	16.0	0.91	1.61	4.65	1.56	1.71
48	16.0	3.43	3.85	3.92	3.72	1.08
49	16.1	1.13	0.84	0.90	0.92	0.81
50	17.1	0.44	0.85	1.26	1.03	2.34
51	3.8	0.37	0.34	0.40	0.49	1.32
52	6.8	0.19	0.15	0.20	0.25	1.32
53	10.9	1.07	0.96	0.72	1.27	1.19
54	11.0	0.71	0.82	0.95	1.30	1.83
55	12.6	1.32	1.40	1.84	2.00	1.52
56	13.7	0.79	1.17	1.22	1.14	1.44
57	13.9	0.91	1.78	1.16	0.93	1.01
58	4.5	0.56	0.70	0.37	0.62	1.11
59	5.4	1.15	0.70	0.95	0.73	0.63
60	9.5	0.30	0.23	0.38	0.44	1.46
61	9.6	0.53	0.45	0.22	0.25	0.47
62	10.0	1.00	0.79	0.66	-	-
63	10.9	0.84	0.73	0.67	0.50	0.60
64	14.4	0.71	0.35	0.92	0.45	0.63
65	15.0	0.91	0.41	1.25	5.19	5.70

66	15.6	0.99	1.80	1.17	3.17	3.20
67	20.0	5.69	5.40	5.70	6.50	1.14
68	26.0	2.48	1.82	3.94	4.16	1.68
69	12.1	1.05	2.22	1.21	1.21	1.15
71	6.3	0.50	1.52	0.49	0.98	1.96
72	8.0	0.33	0.13	0.21	0.10	0.30
73	15.5	1.65	1.04	1.58	1.73	1.05
74	8.3	0.50	0.90	0.68	0.18	0.36
75	7.0	0.79	0.66	0.84	0.64	0.81
76	9.0	0.87	0.65	0.90	1.06	1.22
78	5.0	0.20	-	-	-	-
79	17.2	1.67	1.31	0.95	1.99	1.19
80	14.0	1.46	-	-	-	-

* ND = Not Detectable

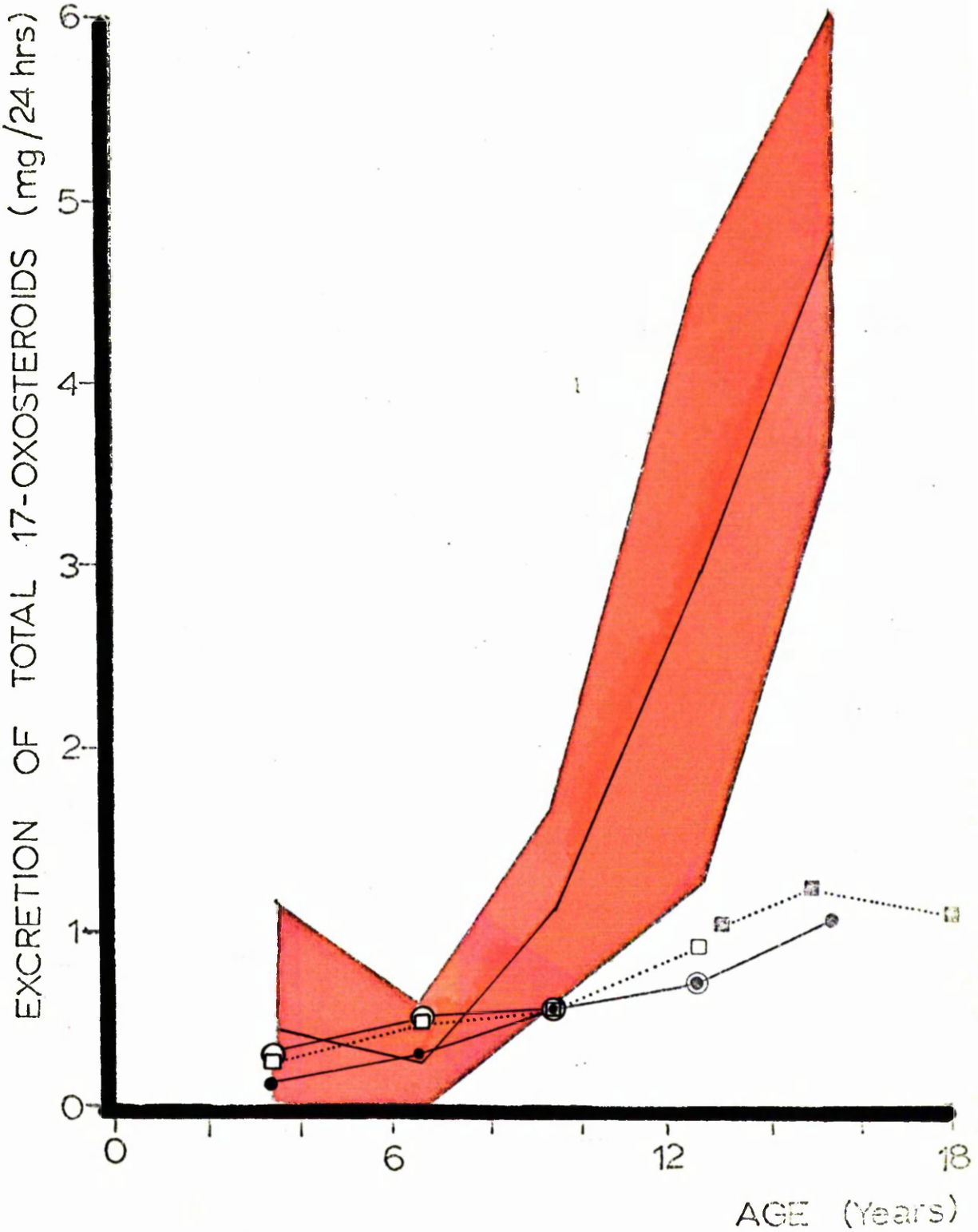


Figure 3.2 The mean daily urinary 17-oxosteroid excretion by patients grouped by age, height and pubertal status. The shaded area represents the mean ± 1 SD daily urinary 17-oxosteroid excretion by normal patients (Prout and Smaith, 1958).

KEY: ⊕ Patients with short stature (height percentiles < 3)
○ Patients with undescended testes
□ Patients with hypogonadism (microgenitalia)
▣ Patients with delayed puberty

children in the early pubertal, pubertal and adolescent age groups (11-17 years) with short stature had a basal 17-oxosteroid excretion well below normal. This difference is statistically highly significant ($p < 0.00005$) and suggests reduced testicular function in patients of short stature.

The basal urinary excretion of 17-oxosteroids by young patients (less than 8 years) with bilateral undescended testes was likewise not significantly different from normal. In the late prepubertal group (8-11 years) the urinary excretion of 17-oxosteroids by these patients was significantly below normal ($0.02 < p < 0.05$) whilst in the early pubertal age group (11-14 years) the basal excretion of 17-oxosteroids was well below normal. This difference is again highly significant ($p < 0.00005$) and indicates reduced testicular output of androgens in patients with undescended testes. No difference was noted in the urinary excretion of 17-oxosteroids between patients with unilateral and bilateral undescended testes.

The basal urinary excretion of 17-oxosteroids by young hypogonadal patients (micropenis, microscrotum with smaller than normal testes) was not significantly different from normal. In the late prepubertal age group (8-11 years) the urinary excretion of 17-oxosteroids by these hypogonadal patients was significantly below normal ($0.01 < p < 0.02$) whilst in the early pubertal age group (11-14 years) the basal excretion of 17-oxosteroids was again very significantly reduced ($p < 0.00005$). These results indicate reduced testicular function and are in agreement with the clinical findings.

The basal urinary excretion of 17-oxosteroids in three groups of patients with delayed puberty (those aged 12-14 years with a pubertal stage of <2; those aged 14-16 years with a pubertal stage of <3 and those aged above 16 years with a pubertal stage of <4) was very significantly reduced ($0.00005 < p < 0.001$).

The urinary excretion of 17-oxosteroids was significantly correlated with age ($r = 0.616$), bone age ($r = 0.596$) and pubertal stage ($r = 0.449$).

The shape of the curves in Figure 3.2 is worthy of note. The normal curve of Prout and Snaith (1958) shows a low 17-oxosteroid excretion for patients below 9 years. This then rises rapidly before puberty and continues to rise through the pubertal and adolescent ages. As the adrenal cortex is the main site of production of the precursors of the urinary 17-oxosteroids before puberty, this rapid rise in the 17-oxosteroid excretion probably represents the phenomenon of 'adrenarche'[1]. By comparison the curves of the 17-oxosteroid excretion in the groups of patients investigated here show no rapid rise in either the prepubertal or pubertal period. It is pertinent, therefore, to comment that whatever the aetiology of the conditions investigated, there would seem to be impaired functioning of both the adrenal and the testis.

The response in 17-oxosteroid excretion to HCG (6,000 I.U. per day) is remarkably variable. Of the 72 patients investigated, the response (columns D, E and F, Table 3.III) was increased, above the basal excretion, in 38 patients (52.78 per cent). No response occurred in 4 patients (5.56 per cent), whilst for the remaining patients (41.66 per cent) there was a diminished excretion below basal levels.

To facilitate further study of the effect of HCG on the various phenotypes, the patients investigated were grouped according to height and pubertal status and the mean stimulation index calculated for each group. As it was considered unethical to give normal children HCG, patients investigated with 'near-normal' phenotype were used for comparison. The results are shown in Table 3.IV.

It will be noted from this table that the excretion of 17-oxosteroids by 'near-normal' patients is approximately trebled after HCG administration. Although patients with short stature had a subnormal increase in the excretion of 17-oxosteroids after HCG stimulation, this was not significantly different from the increase found in the 'near-normal' subjects.

TABLE 3.IV EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON THE 17-OXOSTEROID EXCRETION BY DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)
'Near-normal' phenotype (n = 7)	2.74 ± 0.55
Small stature (n = 31)	1.59 ± 0.53
Unilateral undescended testes (n = 6)	1.26 ± 0.31**
Hypogonadism (n = 23)	1.00 ± 0.12**
Bilateral undescended testes (n = 16)	0.96 ± 0.11**
Delayed puberty (n = 6)	0.62 ± 0.23**

* Stimulation Index = $\frac{\text{Urinary 17-oxosteroid excretion (3rd day)}}{\text{Basal 17-oxosteroid excretion}}$

** Significantly different from 'near-normal' phenotype

Patients with unilateral undescended testes had a subnormal increase in 17-oxosteroid excretion after HCG administration. The rise in 17-oxosteroid excretion in these patients was just significantly below normal ($0.02 < p < 0.05$) but not significantly different from that of patients with bilateral undescended testes. Patients with bilateral undescended testes had no rise in the excretion of 17-oxosteroids following HCG administration. This absence of response to HCG was significantly below normal ($0.01 < p < 0.02$).

Hypogonadal patients (patients with microgenitalia) also showed no response in the 17-oxosteroid excretion to HCG administration. This subnormal response was again significant ($0.02 < p < 0.05$).

Patients with delayed puberty had a decreased urinary excretion of 17-oxosteroids following administration of HCG. This decreased excretion of 17-oxosteroids is very significant ($0.002 < p < 0.005$).

URINARY TESTOSTERONE RESULTS

Several authors have reported normal values for the daily excretion of testosterone in children. Normal values obtained from a review of the literature are shown in Table 3.V. The values obtained in this investigation were compared to the normal values reported by Dalzell and ElAttar (1973). They used a GLC method for the analysis of urine samples.

Many investigators have been unable to detect testosterone in the urine of prepubertal children. Sandberg *et al.* (1964) could not detect urinary testosterone in prepubertal children using either spectrophotometry or gas liquid chromatography with flame ionization detection. Likewise, Eechaute *et al.* (1973), using a fluorimetric method, and Zürbrugg *et al.* (1965), using spectrophotometry, were unable to detect urinary testosterone in prepubertal children. Rosner *et al.* (1965), using spectrophotometry, were only able to detect urinary testosterone in 60 per cent of prepubertal males,

TABLE 3.V DAILY EXCRETION OF TESTOSTERONE IN NORMAL MALE CHILDREN

(µg per 24 hours)

(A Review of the Literature)

Age Range (Years)	Testosterone µg/24 hrs Mean	(Range)	Author(s) (Method of Quantitation)
Prepubertal	< 5		Degenhart et al. (1965) (Double isotope derivative)
Prepubertal	12.2	(3-27.3)	Vermeulen (1966) (GLC - FID)
7-12	3.6	(0-15)	Rosner et al. (1965) (Colorimetry)
17-24	151	± 22	
6-7	< 5		Zurbrügg et al. (1965) (Colorimetry)
5-12		0-2.9	Rudd et al. (1973) (Competitive protein binding)
4-8	0.4	(0.1-0.8)	Vestergaard et al. (1966) (GLC - ECD)
6-13 (3 pubertal)	1.3	(0.4-3.2)	Pal (1971) (Fluorimetry)
9-11		0-14	Gupta (1967) (Colorimetry)
14-18		45-126	Ibayashi et al. (1964) (GLC - FID)
10	1.8		Brooks (1964) (GLC - FID)
Prepubertal	Not detected		Sandberg et al. (1964) (GLC - FID)
13-14		4-180	
4-8	4		Knorr (1967) (Spectrophotometry) Testosterone with epitestosterone
10-11	10		
12	25		
13	35		
14	46		
6-11	6.3		Dalzell and ElAttar (1973) (GLC - Dual Flame Ionization)
11-12	8.2		
13	31.3		
14-16	125.9		
17-19	106.0		
16-20	78.0	(60-103)	Ismail and Harkness (1966) (GLC - FID)
21-63	51.7	(40-64.5)	
14-20	35.9	(25.3-46.5)	Schmidt and Starcevic (1967) (Spectrophotometry)
21-25	60.5	(36.7-84.3)	
10-13	3.4		Morer-Fargas and Nowakowski (1965) (Spectrophotometry)
14	34.6		
15-20	37.9		
21-30	71.7		

and Rudd et al. (1973), using a competitive protein binding assay, could only detect urinary testosterone in 80 per cent of their prepubertal males. However, Vermeulen (1966) detected urinary testosterone in all normal prepubertal children tested and Vestergaard et al. (1966) were able to detect very low levels of testosterone excretion in younger prepubertal children, using electron capture detection following GLC.

Only three groups have published normal results for testosterone excretion throughout the prepubertal, pubertal and adolescent periods. One of these (Knorr, 1967) unfortunately measured combined epitestosterone and testosterone whilst another (Morer-Fargas and Nowakowski, 1965) gives values from aged adult males with only a few prepubertal normal males. I have chosen the results of Dalzell and ElAttar (1973) for comparison of values obtained in this investigation, because their method and mine are most comparable.

The basal urinary excretion of testosterone for patients in this work is shown in column C, Table 3.VI. It will be noted there that the basal urinary excretion of testosterone is low in the majority of the patients, whilst extremely low levels (<1 µg per 24 hours) were found in 23 cases (41.1 per cent). These values of the basal urinary excretion of testosterone were analysed in relation to age, height and pubertal status. Owing to the small number of 'near-normal' subjects here investigated, the values recently published by Dalzell and ElAttar (1973) were used for comparison.

The patients were grouped according to age, height and pubertal status. The mean basal urinary excretion of testosterone of each group was plotted against the mean age of the group. This graph is shown in Figure 3.3.

It will be noted that the shaded area of the graph represents the range ($\pm$ 2 SD) of daily testosterone excretion calculated from the results of Dalzell and ElAttar (1973). This shaded area therefore represents, for the present purposes, the normal range of daily urinary testosterone excretion.

TABLE 3.VI THE EFFECT OF ADMINISTERED HUMAN CHORIONIC GONADOTROPHIN ON THE EXCRETION OF URINARY TESTOSTERONE AND ON THE LEVELS OF PLASMA TESTOSTERONE

Protocol	Age (Years)	Urinary Testosterone (µg/24 hrs)				Plasma Testosterone (ng/100 ml)		Stimulation Index Day 3 / Basal	
		Basal	Day 1	Day 2	Day 3	Basal	Day 3	Urine	Plasma
A	B	C	D	E	F	G	H	I	J
1	0.4	0.22	0.25	0.41	0.78	479	1,886	3.47	3.94
2	2.8	0.19	-	1.51	0.96	501	3,446	5.21	6.88
3	3.3	1.12	-	1.23	1.27	76	77	1.13	1.01
4	10.1	2.31	2.63	5.47	6.88	1,079	15,219	2.98	14.10
5	6.0	0.18	0.18	0.18	0.19	158	283	1.05	1.79
6	12.3	0.31	0.34	0.63	0.77	479	845	2.47	1.76
7	14.8	2.12	2.41	2.59	2.71	225	711	1.28	3.16
8	8.7	0.22	0.37	0.47	0.66	78	289	3.05	3.71
9	14.0	3.62	6.66	8.70	9.87	350	1,166	2.72	3.33
10	8.1	1.00	0.51	1.03	1.35	523	515	1.34	0.98
11	8.5	0.74	0.80	1.00	1.17	103	241	1.57	2.34
12	10.1	0.37	0.39	0.68	1.58	384	535	4.28	1.39
13	11.0	1.41	1.69	1.79	1.95	112	238	1.38	2.13
14	11.5	0.16	0.07	0.17	0.26	-	-	1.63	-
15	12.2	1.93	1.11	6.25	7.14	-	-	3.70	-
16	14.3	3.31	3.83	5.77	7.55	679	853	2.28	1.25
17	15.0	3.37	3.75	4.83	7.57	476	683	2.25	1.44
18	5.4	2.99	2.29	1.03	1.13	95	52	0.38	0.55

19	6.0	4.00	2.58	2.06	1.31	92	74	0.33	0.80
20	8.3	1.41	1.51	1.79	1.92	30	35	1.36	1.17
21	10.1	6.09	6.22	4.36	7.87	37	67	1.29	1.82
22	10.3	3.96	4.07	4.18	4.98	295	309	1.25	1.05
23	11.8	0.22	0.15	0.06	0.21	70	39	0.93	0.56
24	15.0	10.33	6.06	6.03	5.98	327	318	0.58	0.97
25	15.3	12.61	4.87	8.85	-	1,021	666	-	0.65
26	4.1	0.53	0.60	0.74	0.81	75	194	1.52	2.59
27	6.0	0.94	1.04	1.07	1.12	109	303	1.20	2.78
28	7.3	1.04	1.14	1.50	1.55	103	418	1.50	4.06
29	8.2	0.06	0.07	0.08	0.11	16	89	1.83	5.56
30	10.8	4.16	4.56	5.97	8.97	208	634	2.15	3.06
31	10.8	1.83	1.83	2.43	4.34	380	626	2.37	1.65
32	11.0	4.18	4.20	4.34	4.64	224	675	1.11	3.01
33	11.2	0.66	0.87	0.97	1.16	57	161	1.77	2.82
34	11.9	7.96	10.96	11.14	15.32	-	-	1.92	-
35	13.5	-	-	-	-	103	274	-	2.66
36	14.8	1.25	2.54	3.51	4.19	1,031	1,754	3.34	1.70
37	15.0	39.30	-	117.65	133.92	-	-	3.41	-
38	16.1	0.59	0.61	0.61	0.70	309	661	1.17	2.14
39	19.0	3.65	2.25	5.43	6.25	399	1,693	1.71	4.22
40	4.1	-	-	-	-	142	368	-	2.59
41	4.2	6.54	6.68	9.91	12.58	77	467	1.92	6.06

TABLE 3.VI (continued)

Protocol	Age (Years)	Urinary Testosterone (µg/24 hrs)				Plasma Testosterone (ng/100 ml)		Stimulation Index Day 3 / Basal	
		Basal	Day 1	Day 2	Day 3	Basal	Day 3	Urine	Plasma
A	B	C	D	E	F	G	H	I	J
42	13.0	3.96	4.06	4.12	5.12	334	656	1.29	1.96
43	14.0	5.24	7.75	8.11	9.54	276	773	1.82	2.80
44	14.0	15.37	15.81	16.79	18.28	322	705	1.19	2.19
45	16.0	0.14	0.27	0.47	0.56	55	198	3.97	3.60
46	16.0	0.75	0.88	1.11	1.33	304	3,346	1.77	11.01
47	16.0	9.29	10.28	12.38	18.98	299	671	2.04	2.24
48	16.0	3.79	4.68	6.35	6.70	392	540	1.77	1.38
49	16.1	3.02	3.25	4.08	4.94	372	1,417	1.64	3.81
50	17.1	3.03	6.72	13.98	13.86	47	202	4.57	4.30
51	3.8	0.81	0.94	1.78	3.49	157	924	4.31	5.89
52	6.8	1.47	1.85	1.99	2.95	-	-	2.01	-
53	10.9	6.37	6.35	9.79	7.62	857	2,870	1.20	3.35
54	11.0	0.99	-	-	4.27	427	551	4.31	1.29
55	12.6	5.52	4.93	-	20.40	356	1,106	3.70	3.11
56	13.7	-	-	-	-	109	142	-	1.30
57	13.9	0.95	1.78	2.22	2.68	380	2,286	2.81	6.02
58	4.5	1.03	1.09	1.05	1.55	817	857	1.51	1.05
59	5.4	0.29	0.32	0.37	0.46	155	512	1.58	3.30

60	9.5	2.53	2.59	2.65	2.74	50	253	1.08	5.06
61	9.6	1.38	1.51	1.62	3.49	177	1,098	2.54	6.20
62	10.0	0.56	1.88	3.08	-	398	768	-	1.93
63	10.9	0.64	0.58	2.32	2.75	336	992	4.33	2.95
64	14.4	0.72	1.08	1.48	1.94	174	327	2.70	1.88
65	15.0	5.47	5.52	6.41	7.49	376	860	1.37	2.29
66	15.6	9.92	10.64	18.62	19.58	483	1,345	1.97	2.78
67	20.0	14.26	18.81	15.76	19.38	350	840	1.36	2.40
68	26.0	42.85	44.82	61.90	85.52	693	1,841	2.00	2.66
69	12.1	1.33	1.89	4.00	4.42	1,238	11,305	3.32	9.13
70	19.9	3.84	4.76	6.07	8.02	120	335	2.09	2.79
71	6.3	0.81	1.11	1.41	2.96	86	318	3.66	3.70
72	8.0	2.25	0.97	2.12	1.69	317	302	0.75	0.95
73	15.5	1.84	2.07	2.79	3.84	139	520	2.08	3.75
74	8.3	3.25	-	2.41	2.85	-	-	0.88	-
75	7.0	3.14	2.67	6.86	7.43	-	-	2.37	-
76	9.0	4.26	3.37	2.46	2.05	106	248	0.48	2.34
77	9.5	9.80	-	-	-	28,900	-	-	-
78	5.0	22.75	-	-	-	4,452	-	-	-
79	17.2	6.60	-	-	-	40	-	-	-

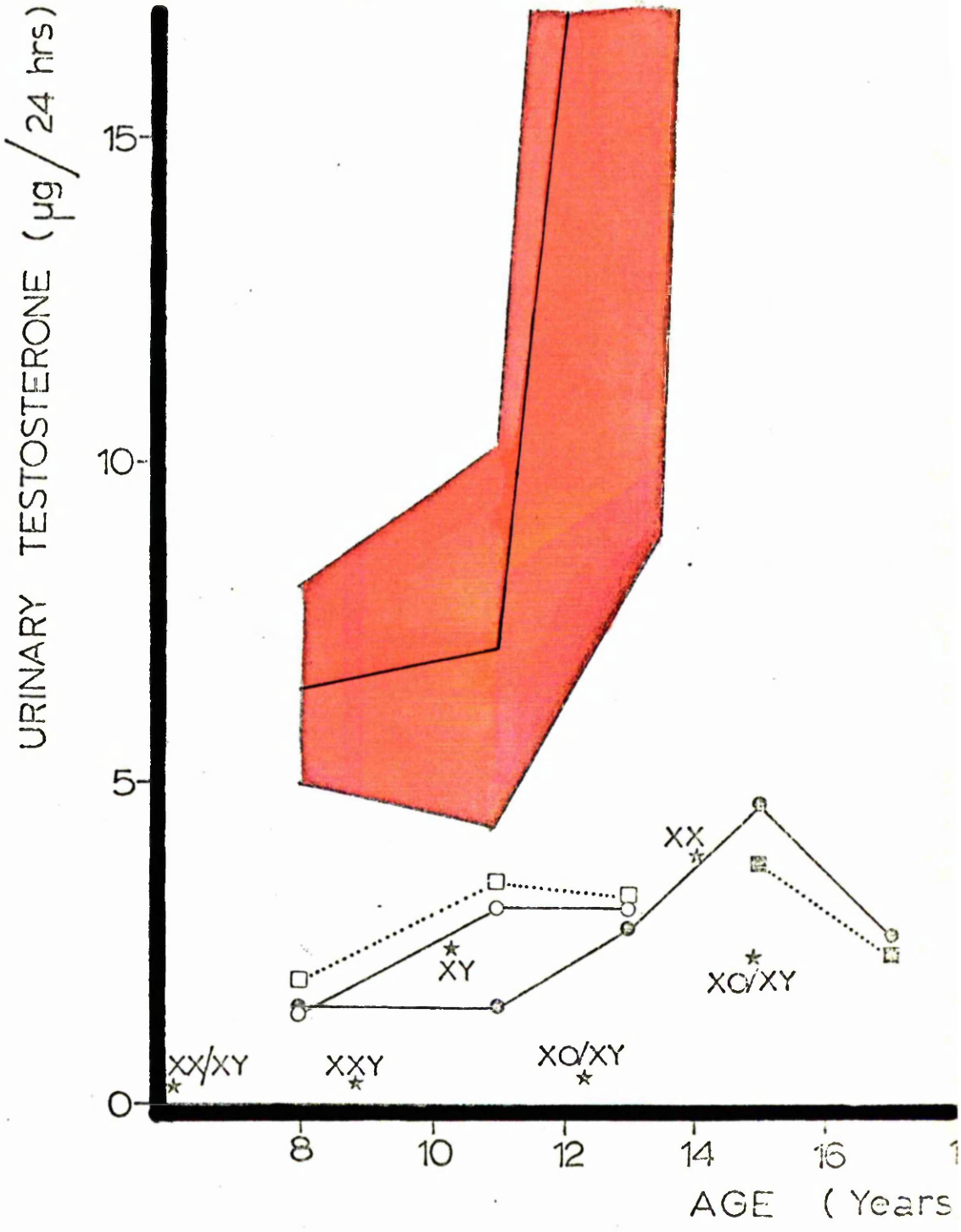


Figure 3.3 The mean basal urinary testosterone excretion by patients grouped by age, height and pubertal status. The shaded area represents the mean ± 2 SD of daily urinary testosterone excretion by normal subjects (Dalzell and ElAttar, 1973).

KEY:
- ★ Patients with abnormal karyotype (protocols 1-9)
- ◉ Patients with short stature (height percentiles < 3)
- ○ Patients with bilateral undescended testes
- □ Patients with hypogonadism (microgenitalia)
- ▣ Patients with delayed puberty

The basal urinary testosterone excretion of all 'near-normal' patients here investigated are within these limits.

The shape of this curve is of interest. There is a low urinary excretion of testosterone for patients less than 12 years which nonetheless increases with age. In early puberty the excretion rate rapidly increases, later to rise very steeply during late puberty to reach adult levels by early adolescence.

The basal urinary excretion of testosterone[1] by patients with short stature was extremely low and there was no rise during puberty. The excretion of urinary testosterone in these patients was significantly below the normal values of Dalzell and ElAttar (1973) during the prepubertal and pubertal age groups ($0.0001 < p < 0.02$) whilst in late puberty and adolescence the urinary testosterone excretion was highly significantly reduced ($p < 0.00005$) owing to the absence of the normal pubertal increase.

The aetiology of the short stature in these patients is known to be due to several factors and although no clinical subdivision is made here these factors will be considered individually later (Chapter 4). Eight of these patients (protocols 10-17) are known to have hGH deficiency and the reduced urinary excretion of testosterone noted in these patients is suggestive of a strong correlation between hGH deficiency and impaired testicular function. However, since the majority of the patients with short stature are not hGH deficient, this indicates that at least part of the dwarfing in these patients may be related to a lack of androgen anabolic stimulus.

The basal daily urinary excretion of testosterone in patients with bilateral undescended testes is likewise subnormal and very similar to that seen in patients with short stature. In prepubertal patients with undescended testes the urinary excretion of testosterone is significantly ($0.00001 < p < 0.05$ below the normal range. In early puberty (12-14 years) this excretion is likewise significantly ($0.02 < p < 0.05$) below normal and significantly

($0.01 < p < 0.02$) below that seen in patients with unilateral undescended testes. The basal urinary excretion of testosterone in patients with unilateral undescended testes is likewise significantly subnormal ($0.002 < p < 0.05$) in all age groups studied and probably reflects the relative testicular hypofunction characteristic of such patients.

The basal urinary excretion of testosterone in patients with hypogonadism (small testes, micropenis, microscotum) is likewise subnormal throughout the prepubertal and pubertal age ranges ($0.00005 < p < 0.05$). The mean urinary excretion of testosterone for these patients (4.12 (range 0.3-14.3) µg per 24 hours) is in good agreement with that found by Moxham and Nabarro (1968) in 11 'hypogonadal' subjects (5.7 (range 1-16.6) µg per 24 hours).

The basal urinary excretion of testosterone in patients with delayed puberty is likewise subnormal, and it is at a prepubertal level. In patients with delayed puberty aged between 14 and 16 years the urinary excretion of testosterone is significantly below normal ($0.002 < p < 0.005$) whilst in patients aged above 16 years this is highly significantly ($p < 0.00005$) subnormal.

Four patients with the testicular feminization syndrome (protocols 1-4) all have a daily urinary excretion of testosterone in the low normal or normal range. The basal urinary excretion is not significantly below normal ($0.05 < p < 0.20$) and probably represents normal production of testosterone by the testes. The basal urinary excretion of testosterone in two patients with Klinefelter's syndrome (XXY karyotype, protocol 8; XX karyotype, protocol 9) are significantly below normal ($0.001 < p < 0.02$) and reflect the hypogonadism characteristic of these patients.

Again, the patients were grouped according to height and pubertal status, and the mean stimulation index calculated for each group. Patients investigated with 'near-normal' phenotype were used for comparison. These indices are shown in Table 3.VII.

TABLE 3.VII THE EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON THE URINARY EXCRETION OF TESTOSTERONE IN PATIENTS WITH DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)
'Near-normal' patients (n = 6)	2.46 ± 0.57
Hypogonadism (n = 24)	1.73 ± 0.26
Small stature (n = 31)	1.74 ± 0.21
Bilateral undescended testes (n = 16)	1.88 ± 0.26
Unilateral undescended testes (n = 6)	2.16 ± 0.68
Delayed puberty (n = 6)	2.30 ± 0.58
Testicular feminization syndrome (n = 4)	3.18 ± 0.81

* Stimulation Index = $\frac{\text{Urinary excretion of testosterone (3rd day)}}{\text{Basal urinary excretion of testosterone}}$

It will be noted from this table that the urinary excretion of testosterone by 'near-normal' patients in the investigation is increased two-and-a-half fold after HCG stimulation. Patients with other phenotypes have similar stimulation indices, not significantly different ($0.30 < p < 0.90$) from that of 'near-normal' patients. However, it is interesting that the stimulation of urinary testosterone following HCG administration in patients with hypogonadism is slightly lower than normal, whilst in patients with testicular feminization syndrome this stimulation index is slightly above normal. This high index probably reflects either the Leydig cell hyperplasia noted in this syndrome or the fact that testosterone is not metabolized by the tissues and therefore more is available for excretion unchanged.

PLASMA TESTOSTERONE RESULTS

Normal values for plasma testosterone in children, as reported in the literature, are given in Table 3.VIII. The results obtained in this investigation were compared to the normal values of Frasier et al. (1969) which they obtained using a double isotope dilution method of quantitation.

Reported levels of plasma testosterone in prepubertal children are very low and methods for testosterone quantitation must therefore be very sensitive. Thus Eechaute et al. (1973), using a fluorimetric method, and Sandberg et al. (1964), using flame ionization detection after gas-liquid chromatography, were unable to detect plasma testosterone in prepubertal males. Similarly, the levels detected by Degenhart et al. (1965), using a double isotope derivative technique, and Zurbrügg et al. (1965), using colorimetry, were below the sensitivity of the method. Dessypris and Adlercreutz (1972) and Rudd et al. (1973), using a competitive protein binding assay, could only quantitate plasma testosterone in some 75 per cent of the normal prepubertal children tested.

Van der Molen and Groen (1967), using electron capture detection of testosterone chloroacetate after gas-liquid chromatography, found low levels

TABLE 3.VIII PLASMA LEVELS OF TESTOSTERONE IN NORMAL MALE CHILDREN AND ADULTS (ng per 100 ml)

(A Review of the Literature)

Age Range (Years)	Testosterone ng/100 ml Mean (Range)	Authors (Method of Quantitation)
Prepubertal	16 (0-36)	Dessypris and Adlercreutz (1972) (Competitive protein binding)
5-13	0-40	Rudd et al. (1973) (Competitive protein binding)
2-14	31.8 ± 10	Saez and Bertrand (1968) (Double isotope dilution)
0.3-12	25	Forest and Migeon (1970)
3-8 days	13.8 ± 7.2	Forest et al. (1972) (Radioimmunoassay)
1-10	6.58 ± 2.48	
Adult	565 ± 146	
1-10	15	Van der Molen and Groen (1967) (GLC - ECD)
10-16	170	
22-59	680	
4-10	42 ± 9	Frasier and Horton (1966) (Double isotope dilution)
2-5	8	Gandy and Peterson (1968) (Double isotope dilution)
5-8	13	
8-10	34	
10-12	75	
12-14	243	
14-15	643	
8	5	Wieland et al. (1970) (Competitive protein binding)
9	13	
10	43	
11	94	
12	124	
14	604	
5-9	18 ± 4	Winter et al. (1972) (Competitive protein binding)
9-11	33 ± 6	
11-14	95 ± 27	
14-16	287 ± 57	
16-20	472 ± 50	
20-22	534 ± 36	
4-10	38	Frasier et al. (1969) (Double isotope dilution)
10-12	77	
12-13	262	
13-14	235	
14-15	327	
15-16	494	
Adult	660 ± 80	

of plasma testosterone in prepubertal children. They noted that levels in older prepubertal children (above 10 years) were, however, greater. Further work by Gandy and Peterson (1968), using a double isotope dilution technique, gave similar low levels for children in the prepubertal age group. They found the levels of plasma testosterone began to rise between the ages of 10 to 12 years and they correlated this with the increase in the size of the testes and vascularity of the penis which develop at this age. They found a further, greater rise in plasma testosterone at puberty and noted that adult levels were reached by the time of 'young adolescence'.

Frasier et al. (1969), using a double isotope dilution technique, also noted this prepubertal rise and the rapid rise at puberty, although they found plasma testosterone in young adolescents to be below adult levels. Likewise, Winter et al. (1972), using a similar method of quantitation, studied plasma testosterone levels in the prepubertal, pubertal, adolescent and adult age ranges, and correlated these to the stage of pubertal development They noted the late prepubertal rise and related this rise to early testicular enlargement. They also found the large pubertal rise in plasma testosterone and found that adult levels were not reached below the age of 20 years.

Forest et al. (1972) studied plasma testosterone measured by a radioimmunoassay technique, in neonates and in prepubertal males. They found higher levels just after birth (3-8 days) than in the prepubertal age. They also noted no significant difference between plasma testosterone levels in males and females in the prepubertal age.

August et al. (1972) correlated plasma testosterone with pubertal development. They found low prepubertal levels and noted that the late prepubertal rise was correlated with early somatic pubertal changes. Levels of plasma testosterone at puberty and during adolescence were similar to those found by Winter et al. (1972).

The basal level of plasma testosterone is shown in column G, Table 3.VI. These values of plasma testosterone were grouped according to patient age,

height and pubertal status. The mean basal level of plasma testosterone of each group was plotted against the mean age of the group. This graph is shown in Figure 3.4. Owing to the small number of 'near-normal' subjects investigated, the values published by Frasier et al. (1969) were used for comparison following similar grouping.

It will be noted that the shaded area of this graph represents the range ($\pm$ 2 SD) of plasma testosterone calculated from the results of Frasier et al. (1969). This shaded area therefore represents, for the present purposes, the normal range of plasma testosterone. The basal level of all 'near-normal' patients here investigated is within this range.

The shape of this curve is of interest. There is a very low level of plasma testosterone in patients less than 11 years of age. This then increases rapidly throughout early puberty, puberty and adolescence to reach adult levels by approximately 20 years of age. The initial prepubertal rise in peripheral plasma testosterone concentration is consistent with the clinical observations that testicular size and vascularity of the penis also increase at this age.

The peripheral plasma concentration of testosterone in patients of short stature in this investigation (Figure 3.4) rose slowly from early childhood to adolescence. Although the mean plasma testosterone level was seemingly high in the younger age groups, the basal plasma testosterone in these patients was not significantly different ($0.20 < p < 0.90$) from the normal results of Frasier et al. (1969) at each age group measured.

The plasma concentration of testosterone in patients with bilateral undescended testes was quite variable. In patients aged between 4 and 12 years the plasma testosterone concentration was not significantly different from normal, whilst in the oldest patient with bilateral undescended testes (protocol 69, 12.1 years) the plasma testosterone concentration was significantly raised (1,238 ng per 100 ml; $p < 0.0001$).

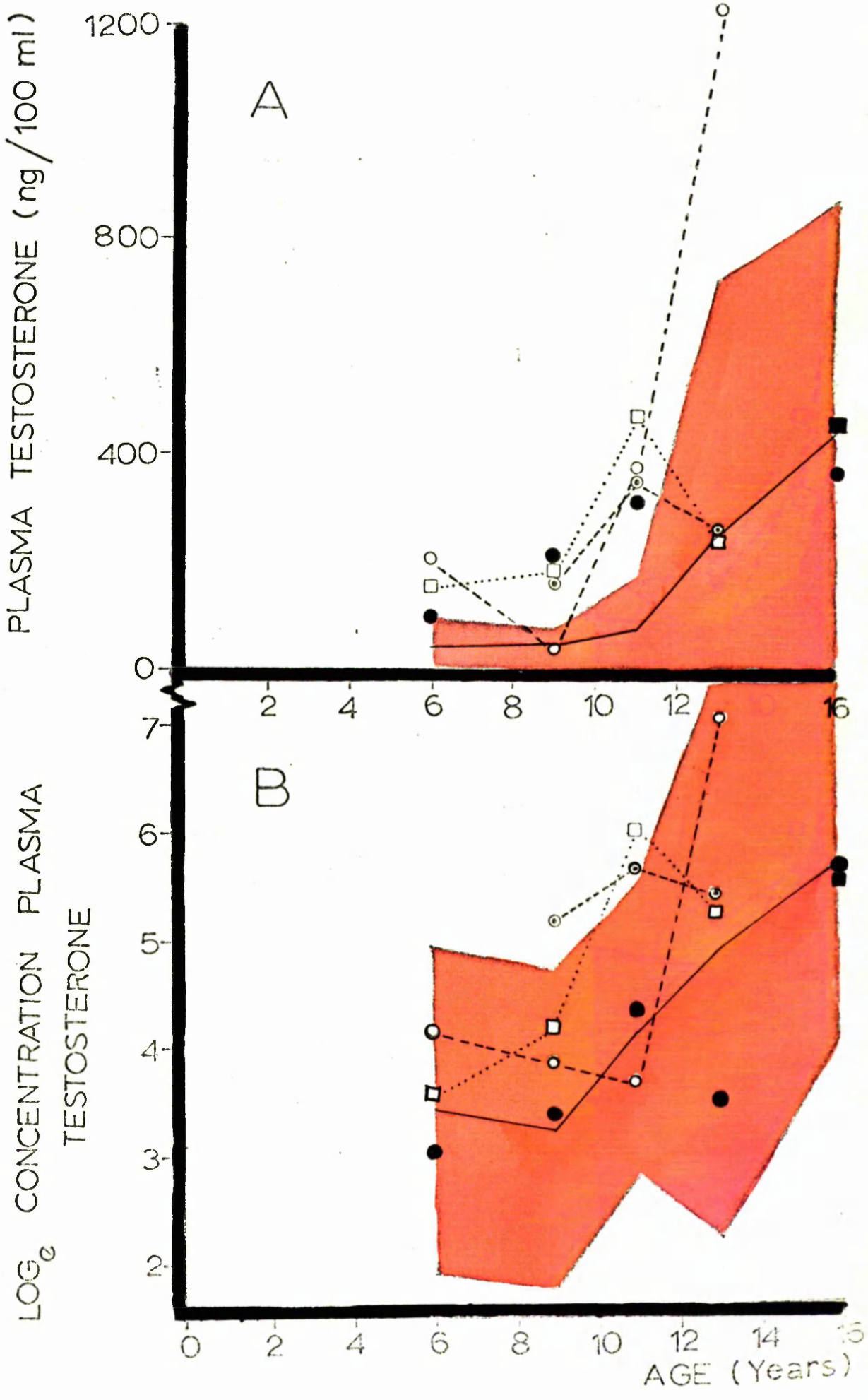
Figure 3.4

(A) The mean basal plasma testosterone concentration in patients grouped by age, height and pubertal status. The shaded area represents the mean $\pm$ 2 SD plasma testosterone concentration in normal subjects (Frasier et al., 1969).

(B) As figure 3.4 A but following logarithmic transformation of results, to normalize the skewed distribution.

KEY:

- ⊛ Patients with short stature (height percentile <3)
- ○ Patients with bilateral undescended testes
- ⊙ Patients with unilateral undescended testes
- □ Patients with hypogonadism (microgenitalia)
- ▩ Patients with delayed puberty


A
B
PLASMA TESTOSTERONE (ng/100 ml)
LOG$_e$ CONCENTRATION PLASMA TESTOSTERONE
AGE (Years)
1200
800
400
0
7
6
5
4
3
2
0
2
4
6
8
10
12
14
16

The plasma testosterone concentration in patients with unilateral undescended testes was also variable. In patients below 12 years the plasma testosterone levels were significantly raised ($0.0002 < p < 0.005$) whilst in older patients (above 12 years old) the plasma testosterone concentration was similar ($p > 0.80$) to the normal levels of Frasier et al. (1969).

Raised plasma testosterone concentration in patients with undescended testes may arise from increased testosterone production by the cryptorchid testes owing to the increased ambient intra-abdominal temperature or to the change in Leydig cells as described by Clegg (1961). However, as many of these patients have additional clinical features of hypogonadism such as micropenis and microscrotum, it is probable, whatever the aetiology of the elevated plasma testosterone concentration, that there is either an increased binding of testosterone in the plasma by testosterone binding globulin, thus preventing testosterone liberation, or there may be tissue insensitivity to testosterone. This latter might result in less degradation of circulating testosterone.

The plasma concentration of testosterone in patients with hypogonadism was similarly very variable. In young patients (4-10 years) the plasma testosterone levels were not significantly different from normal ($0.20 < p < 0.80$). Patients aged between 10 and 12 years with hypogonadism had a significantly raised plasma testosterone concentration ($0.0002 < p < 0.0005$ However, the expected rise in plasma testosterone concentrations did not occur at normal pubertal age and the plasma testosterone levels were then in the normal range. Again, it is probable that there could be either a tissue insensitivity to testosterone or an increased binding of testosterone in plasma in these hypogonadal patients who actually have adequate testosterone production.

As with older patients with hypogonadism, the mean plasma level of testosterone in patients with delayed puberty was not significantly different from normal ($p > 0.50$).

Again, the patients were grouped according to height and pubertal status and the mean stimulation index calculated for each group. Again, patients investigated with 'near-normal' phenotype were used for comparison. These indices are shown in Table 3.IX.

It will be noted from this table that the peripheral plasma concentration of testosterone in 'near-normal' patients in this investigation is increased approximately two-and-a-half fold following HCG administration. Patients with other phenotypes have similar stimulation indices not significantly different ($0.20 < p < 0.90$) from that of 'near-normal' patients. However, it is interesting that the stimulation of plasma testosterone concentrations following HCG administration is above that of 'near-normal' patients in all phenotypes with the exception of that of patients with delayed puberty. It is also interesting that the highest rise in testosterone concentrations following HCG is noted in patients with the testicular feminization syndrome. Again, the good increase in plasma testosterone concentrations seen in these patients following HCG administration is consistent with the Leydig cell hyperplasia often noted in association with this syndrome or the fact that testosterone is not metabolized by the tissues.

URINARY ANDROGEN METABOLITES

The excretion of urinary androgen metabolites was studied in all of the 76 patients investigated. In two patients the study was repeated following treatment with HCG and HMG for one month. Complete results for individual patients will be found in a later section of this thesis (Chapter 5). To facilitate presentation of these results, individual steroids and groups of steroids (11-deoxy-17-oxosteroids, the 11-oxo-17-oxosteroids and the dihydroxysteroids) will be considered separately.

TABLE 3.IX THE EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON THE PLASMA CONCENTRATION OF TESTOSTERONE IN PATIENTS WITH DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)
'Near-normal' phenotype (n = 4)	2.53 ± 0.11
Small stature (n = 31)	2.53 ± 0.42
Hypogonadism (n = 24)	2.75 ± 0.41
Bilateral undescended testes (n = 16)	2.67 ± 0.58
Unilateral undescended testes (n = 6)	3.04 ± 0.69
Delayed puberty (n = 6)	2.41 ± 0.50
Testicular feminization syndrome (n = 4)	6.48 ± 2.81

* Stimulation Index = $\frac{\text{Plasma concentration of testosterone (3rd day)}}{\text{Basal plasma concentration of testosterone}}$

URINARY 11-DEOXY-17-OXOSTEROIDS

The daily excretion values for androsterone, aetiocholanolone and dehydroepiandrosterone are shown in Table 3.X. Corresponding values for epiandrosterone (3β-hydroxy-5α-androstane-17-one) will be considered separately (Table 3.XVI). In columns C, E and G (Table 3.X) are shown the basal daily excretion rates of androsterone, aetiocholanolone and dehydroepiandrosterone respectively. These basal levels are very variable even for patients of similar age and phenotype (cf protocols 45 and 49). Generally, the daily excretion of androsterone was found to be much greater than that for aetiocholanolone. This, however, is the normal prepubertal pattern of excretion for these two steroids. The daily excretion of dehydroepiandrosterone is extremely low (< 10 μg per 24 hours) in 11 patients and was not detected in a further 7.

On stimulation with HCG, the daily urinary output of these 11-deoxy-17-oxosteroids was also noted to be very variable. However, the post-stimulation daily excretion of androsterone, aetiocholanolone and dehydroepiandrosterone in the 'near-normal' patients was not significantly different from and closely paralleled the rise in the daily excretion of the urinary 17-oxosteroids as assayed by the Zimmermann reaction.

To facilitate further analysis of the urinary 11-deoxy-17-oxosteroid excretion rates the values found in this study were compared with those reported by Vestergaard (1965), Paulsen *et al.* (1966) and Tanner and Gupta (1968) obtained for normal children and adolescents. These normal values are shown in Table 3.XI.

Androsterone

The patients were grouped according to age, height and pubertal status. The basal daily excretion of androsterone (column C, Table 3.X) for each group was plotted against the mean age of the group. This graph is shown in Figure 3.5.

TABLE 3.X DAILY URINARY EXCRETION OF INDIVIDUAL 11-DEOXY-17-OXOSTEROIDS AND THE RESPONSE TO ADMINISTERED HCG (µg/24 hrs)

Protocol	Age (Years)	Androsterone		Aetiocholanolone		Dehydroepiandrosterone		$\frac{A}{Ae}$ Basal	$\frac{A}{Ae}$ Day 3
		Basal	Day 3	Basal	Day 3	Basal	Day 3		
A	B	C	D	E	F	G	H	I	J
1	0.4	19	13	8	16	23	128	2.38	0.81
2	2.8	152	277	56	97	ND*	ND	2.71	2.86
3	3.3	133	206	16	63	80	95	8.31	3.27
4	10.1	150	272	79	125	19	44	1.90	2.18
5	6.0	11	13	1	12	1	3	11.00	1.08
6	12.3	467	761	327	717	157	77	1.43	1.06
7	14.8	418	488	153	153	146	172	2.73	3.08
8	8.7	52	235	7	76	11	59	7.43	3.09
9	14.0	268	751	70	269	128	332	3.82	2.79
10	8.1	128	58	16	22	28	18	8.00	2.64
11	8.5	54	613	35	234	13	41	1.54	2.62
13	11.0	18	10	6	12	1	3	3.00	0.83
14	11.5	4	57	2	30	2	4	2.00	1.90
15	12.2	359	481	199	161	48	55	1.80	2.99
16	14.3	111	192	95	165	50	48	1.17	1.19
17	15.0	96	487	199	626	202	281	0.48	0.78
18	5.4	42	97	2	1	4	7	21.00	97.00
19	6.0	28	8	19	1	4	2	1.47	8.00
20	8.3	298	362	54	73	701	195	5.52	4.96
21	10.1	126	215	82	360	17	255	1.54	0.60

22	10.3	146	161	66	103	36	9	2.21	1.56
23	11.8	705	404	147	92	ND	ND	4.80	4.39
24	15.0	141	190	109	153	31	11	1.29	1.24
25	15.3	135	-	80	-	251	-	1.69	-
26	4.1	16	39	1	4	3	4	16.00	9.75
27	6.0	1	2	1	1	40	9	1.00	2.00
28	7.3	9	30	4	1	1	41	2.25	30.00
29	8.2	7	16	4	2	1	5	1.75	8.00
30	10.8	186	475	138	370	25	32	1.35	1.28
31	10.8	285	479	129	178	57	40	2.21	2.69
32	11.0	250	154	108	69	50	43	2.31	2.23
33	11.2	169	195	89	99	12	21	1.90	1.99
34	11.9	114	129	413	520	244	110	0.28	0.25
35	13.5	166	292	111	163	25	35	1.50	1.79
36	14.8	50	124	36	109	5	8	1.39	1.14
37	15.0	397	582	481	583	38	61	0.83	1.00
38	16.1	31	226	23	215	3	4	1.35	1.05
39	19.0	478	872	336	539	69	89	1.42	1.62
40	4.1	79	124	10	33	67	66	7.90	3.76
41	4.2	7	17	23	13	4	3	0.30	1.31
42	13.0	79	501	63	406	5	15	1.25	1.24
43	14.0	121	588	218	576	25	38	0.56	1.02
44	14.0	152	501	117	412	53	128	1.30	1.22
45	16.0	74	260	61	252	5	145	1.21	1.03

TABLE 3.X (continued)

Protocol	Age (Years)	Androsterone		Aetiocholanolone		Dehydroepiandrosterone		$\frac{A}{Ae}$ Basal	$\frac{A}{Ae}$ Day 3
		Basal	Day 3	Basal	Day 3	Basal	Day 3		
A	B	C	D	E	F	G	H	I	J
46	16.0	113	567	58	533	32	272	1.95	1.06
47	16.0	61	565	62	937	272	161	0.98	0.60
48	16.0	14	535	14	314	18	190	1.00	1.70
49	16.1	559	916	312	378	77	9	1.79	2.42
50	17.1	250	623	151	578	13	77	1.66	1.08
51	3.8	56	107	40	86	170	5	1.40	1.24
52	6.8	96	217	81	101	7	108	1.19	2.15
53	10.9	107	244	45	76	ND	ND	2.38	3.21
54	11.0	755	1,005	33	93	96	94	22.88	10.81
55	12.6	705	405	597	221	386	64	1.18	1.83
56	13.7	609	1,433	379	981	499	823	1.61	1.46
57	13.9	162	229	53	98	98	225	3.06	2.34
58	4.5	17	36	20	21	ND	ND	0.85	1.71
59	5.4	3	9	15	28	3	11	0.20	0.32
60	9.5	15	67	8	20	4	222	1.88	3.35
61	9.6	56	99	35	74	72	137	1.60	1.34
62	10.0	23	-	10	-	ND	-	2.30	-
63	10.9	100	137	60	129	229	242	1.67	1.06
64	14.4	157	326	60	164	35	113	2.62	1.99
65	15.0	505	732	715	1,182	128	298	0.71	0.62

66	15.6	486	1,200	188	585	92	90	2.59	2.05
67	20.0	1,455	2,191	1,695	2,350	79	1,327	0.86	0.93
68	26.0	2,326	3,521	1,470	2,004	446	501	1.58	1.76
69	12.1	444	250	169	363	ND	ND	2.63	0.69
70	19.9	1,498	2,703	444	1,410	189	408	3.37	1.92
71	6.3	34	38	7	7	10	9	4.86	3.43
72	8.0	194	103	7	19	31	27	27.71	5.42
73	15.5	426	693	355	676	87	76	1.20	1.03
74	8.3	70	106	27	49	7	6	2.60	2.16
75	7.0	60	41	10	19	ND	ND	6.00	2.16
76	9.0	316	426	79	116	28	11	4.00	3.67
77	9.5	1,439	-	128	-	364	-	11.24	-
78	5.0	325	-	58	-	5	-	5.60	-
79	17.2	281	219	242	206	40	36	1.16	1.06
80	14.0	933	745	421	417	502	792	2.22	1.79

* ND = Not Detectable

TABLE 3.XI EXCRETION OF INDIVIDUAL 11-DEOXY-17-OXOSTEROIDS IN NORMAL CHILDREN AT VARIOUS AGES*

Age Range (years)	Androsterone (µg/24 hrs)	Aetiocholanolone (µg/24 hrs)	Dehydroepiandrosterone (µg/24 hrs)
3-6	143 ± 76 n = 8	48 ± 34 n = 5	21 ± 25 n = 8
6-9	290 ± 163 n = 16	172 ± 105 n = 13	57 ± 56 n = 16
9-13	418 ± 302 n = 42	253 ± 161 n = 42	101 ± 98 n = 42
13-16	1235 ± 408 n = 6	925 ± 531 n = 6	225 ± 146 n = 6
16+	3950 ± 240 n = 2	2975 ± 700 n = 2	1490 ± 1485 n = 2

* Mean ± 1 standard deviation calculated from combined results of Vestergaard (1965), Paulsen *et al.* (1966), and Tanner and Gupta (1968)

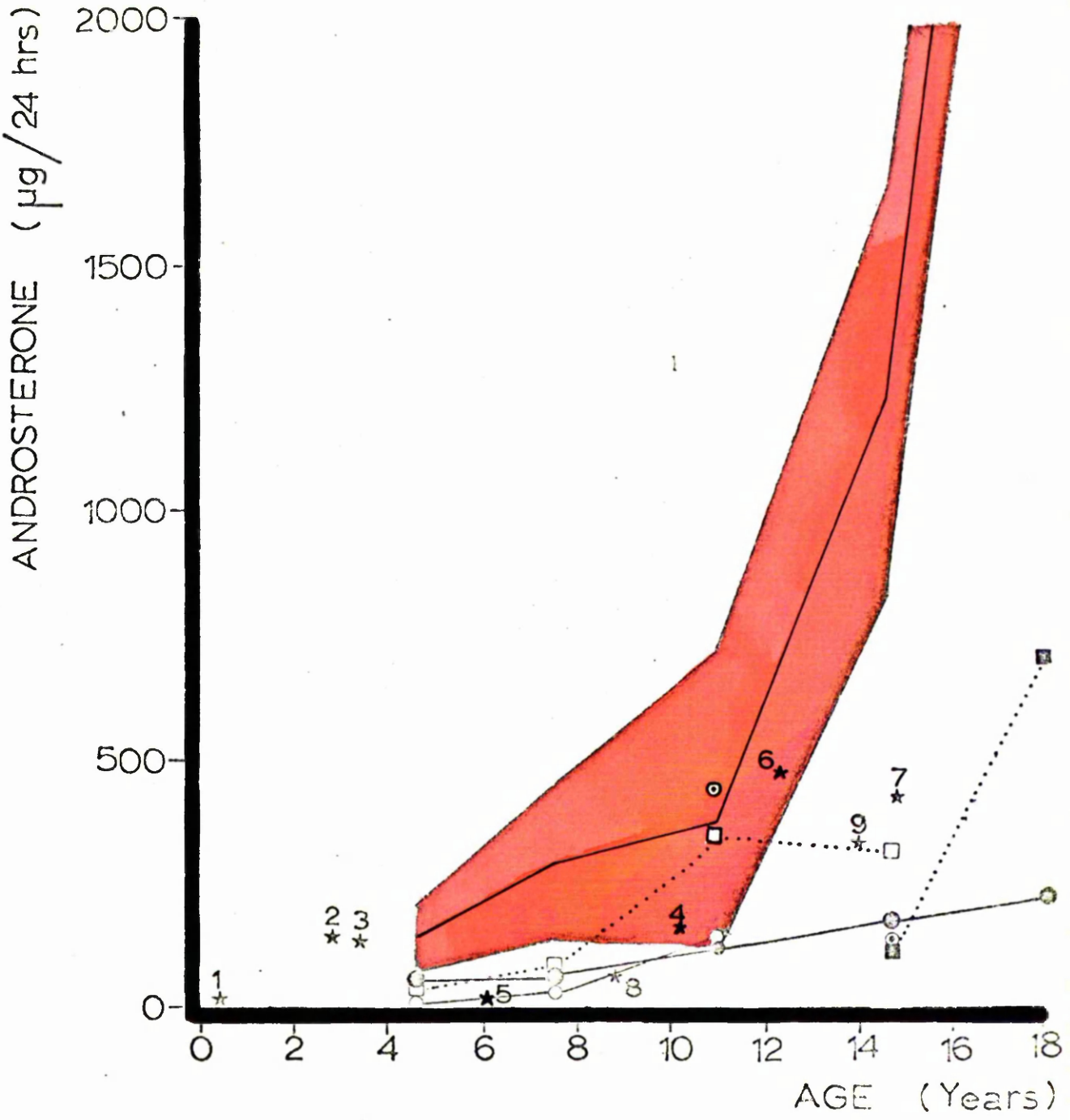


Figure 3.5 The mean basal urinary androsterone excretion by patients grouped by age, height and pubertal status. The shaded area represents the mean $\pm$ 1 SD of daily androsterone excretion by normal subjects as shown in Table 3.XI.

KEY:

- Patients with short stature
- Patients with bilateral undescended testes
- Patients with unilateral undescended testes
- Patients with hypogonadism (microgenitalia)
- Patients with delayed puberty
- Patients with abnormal karyotype (identified by protocol number)

It will be noted that the shaded area of the graph represents the range ($\pm$ 1 SD) of daily androsterone excretion calculated from the combined results of Vestergaard (1965), Paulsen et al. (1966) and Tanner and Gupta (1968), as shown in Table 3.XI. This shaded area therefore represents, for the present purposes, the normal range of the daily androsterone excretion. The basal daily androsterone excretion of all 'near-normal' patients here investigated are within these limits.

The shape of this curve is of interest. There is a low, but increasing, daily excretion of androsterone for patients less than 9 years. This then rises more rapidly before puberty and at puberty there is a very steep rise in the daily androsterone excretion which then continues through adolescence to adulthood. As the adrenal cortex is the main site of production of androsterone before puberty, the initial prepubertal rise in androsterone excretion probably represents the phenomenon of 'adrenarche', whilst the rapid pubertal rise in the daily androsterone excretion is probably more correlated to the increase in testicular secretion of precursors of androsterone.

It will be noted from Figure 3.5 that the basal daily excretion of androsterone in patients of short stature is very low and continues to rise at the prepubertal rate, there being neither adrenal nor testicular correlated rise. Below 6 years of age the basal daily excretion of androsterone in these patients, although very low, is not significantly different from the normal value ($0.05 < p < 0.10$). In older prepubertal children of short stature (6-13 years) the daily excretion of androsterone is significantly below the normal range ($0.0002 < p < 0.005$) whilst in the pubertal and adolescent age groups (13-20 years) this difference is statistically highly significant ($0.00005 < p < 0.0005$). Reduced testicular and/or adrenal function must be concluded.

The basal daily excretion of androsterone in patients with bilateral undescended testes is likewise subnormal. Below 6 years of age, the daily

excretion of androsterone in these patients is extremely small (< 20 μg per 24 hours) this being statistically significantly below normal ($0.002 < p < 0.005$). In older prepubertal children with bilateral undescended testes (6-13 years) the daily excretion of androsterone is significantly below normal ($0.001 < p < 0.01$) and reduced testicular function must be concluded. Furthermore as no difference was found between the daily androsterone excretion of a patient with bilateral undescended testes (protocol 26, Table 3.X) and his two brothers following successful bilateral orchidopexy (protocols 27 and 28, Table 3.X) it must be concluded that although orchidopexy improves the spermatogenic potential of the testes abnormal steroidogenesis still persists.

The basal urinary excretion of androsterone in patients with hypogonadism (small external genitalia) is very low. Below 9 years of age the daily excretion of androsterone by these patients is significantly below normal ($0.002 < p < 0.005$). In older prepubertal children the daily urinary excretion of androsterone is within the normal range whilst in hypogonadal patients of pubertal age (13-16 years) absence of a pubertal rise made this excretion significantly subnormal ($0.01 < p < 0.02$).

The basal urinary excretion of androsterone in a group of patients with delayed puberty (aged 13-16 years) is highly significantly below the normal range ($0.0005 < p < 0.001$). It is interesting and perhaps of clinical significance that this group of patients has neither adrenal nor pubertal correlated rises, whilst an older group of patients with delayed puberty had an adrenal but not a testicular correlated rise. The urinary androsterone excretion in this group of older patients with delayed puberty was not significantly reduced ($0.05 < p < 0.10$).

Two patients with the Prader-Willi syndrome (dwarfism, hypogonadism and obesity) (protocols 32 and 72, Table 3.X) had daily urinary androsterone levels within the low normal level for patient age, whilst one patient with

this syndrome and bilateral undescended testes had a subnormal urinary level of androsterone significantly below the normal level and equivalent to that found in patients with bilateral undescended testes. Similarly, a patient with the Laurence-Moon-Biedl syndrome with bilateral undescended testes had a basal daily urinary androsterone level equivalent to that found in patients with bilateral undescended testes.

Four patients with the testicular feminization syndrome (protocols 1-4) all had daily androsterone excretion values within the normal range. The basal daily androsterone level in two patients with Klinefelter's syndrome (protocols 8 and 9), although both below the normal range, were not significantly different ($0.10 < p < 0.25$) from normal. Similarly, the basal daily androsterone levels found in two patients with the XO/XY syndrome were within the lower normal range, whilst the basal daily urinary androsterone level in one patient with the XX/XY syndrome (protocol 5) was significantly below normal ($0.02 < p < 0.05$).

The urinary excretion of androsterone was significantly correlated with age ($r = 0.519$), bone age ($r = 0.610$) and pubertal stage ($r = 0.546$).

To facilitate further study of the effect of HCG the patients were grouped according to height and pubertal status and the mean stimulation index calculated for each group. Patients investigated with 'near-normal' phenotype were used for comparison. These indices are shown in Table 3.XII.

It will be noted from this table that the urinary excretion of androsterone by 'normal' patients in this investigation is increased 1.6 fold following HCG stimulation. Patients of small stature (height percentile <3), having a very low basal urinary androsterone excretion, were found to have a very good increase in the urinary androsterone excretion after HCG stimulation. This was significantly above ($0.02 < p < 0.05$) the 'normal' stimulation values found in this investigation.

Patients with undescended testes, with hypogonadism or with delayed puberty, all had a good increase in the urinary androsterone excretion

TABLE 3.XII EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON THE ANDROSTERONE EXCRETION BY DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)
'Near-normal' phenotype (n = 7)	1.63 ± 0.19
Short stature (n = 31)	2.99 ± 0.61**
Bilateral undescended testes (n = 16)	2.58 ± 0.83
Hypogonadism (n = 24)	2.18 ± 0.44
Unilateral undescended testes (n = 6)	2.07 ± 0.88
Delayed puberty (n = 6)	1.92 ± 0.47
Testicular feminization syndrome (n = 4)	1.62 ± 0.32
XO/XY syndrome (n = 2)	1.39 ± 0.30
Klinefelter's syndrome (XXY, XX) (n = 2)	3.56 ± 0.94**
XX/XY syndrome (n = 1)	1.18

* Stimulation Index = $\frac{\text{Urinary androsterone excretion (3rd day)}}{\text{Basal androsterone excretion}}$

** Significantly above normal

after HCG stimulation from very low basal values. The stimulation of androsterone excretion in these patients was not statistically above that of the 'normal' patients investigated here. Thus it would appear that the primary failure in these patients is one of gonadotrophin lack and not testicular failure as such.

Four patients with the testicular feminization syndrome have a normal increase in urinary androsterone excretion, after HCG, from normal basal levels. It is interesting that in two patients with Klinefelter's syndrome (protocols 8 and 9) with low normal basal urinary androsterone excretion, the response to HCG is significantly above normal ($0.00005 < p < 0.0001$). In two patients with the XO/XY syndrome, having basal urinary androsterone excretions in the low normal range, the response to HCG stimulation is within the normal range. Similarly, a patient with the XX/XY genotype (protocol 5) has a normal response to HCG stimulation although the basal level is significantly below normal. Definite conclusions cannot be given here but these results will be further discussed later.

Aetiocholanolone

Again, the patients investigated were grouped according to age, height and pubertal status. The basal urinary excretion of aetiocholanolone (column E, Table 3.X) for each group was plotted against the mean age of the group. The graph is shown in Figure 3.6.

It will be noted that the shaded area of the graph represents the range (mean $\pm$ 1 SD) of the daily urinary aetiocholanolone excretion calculated from the combined results of Vestergaard (1965), Paulsen *et al.* (1966) and Tanner and Gupta (1968), as shown in Table 3.XI. This shaded area therefore represents the normal urinary aetiocholanolone excretion. The shape of this curve closely resembles that for the urinary androsterone excretion. Again, there is a low, but increasing, aetiocholanolone excretion rate during childhood. Between 11 years and puberty there is a

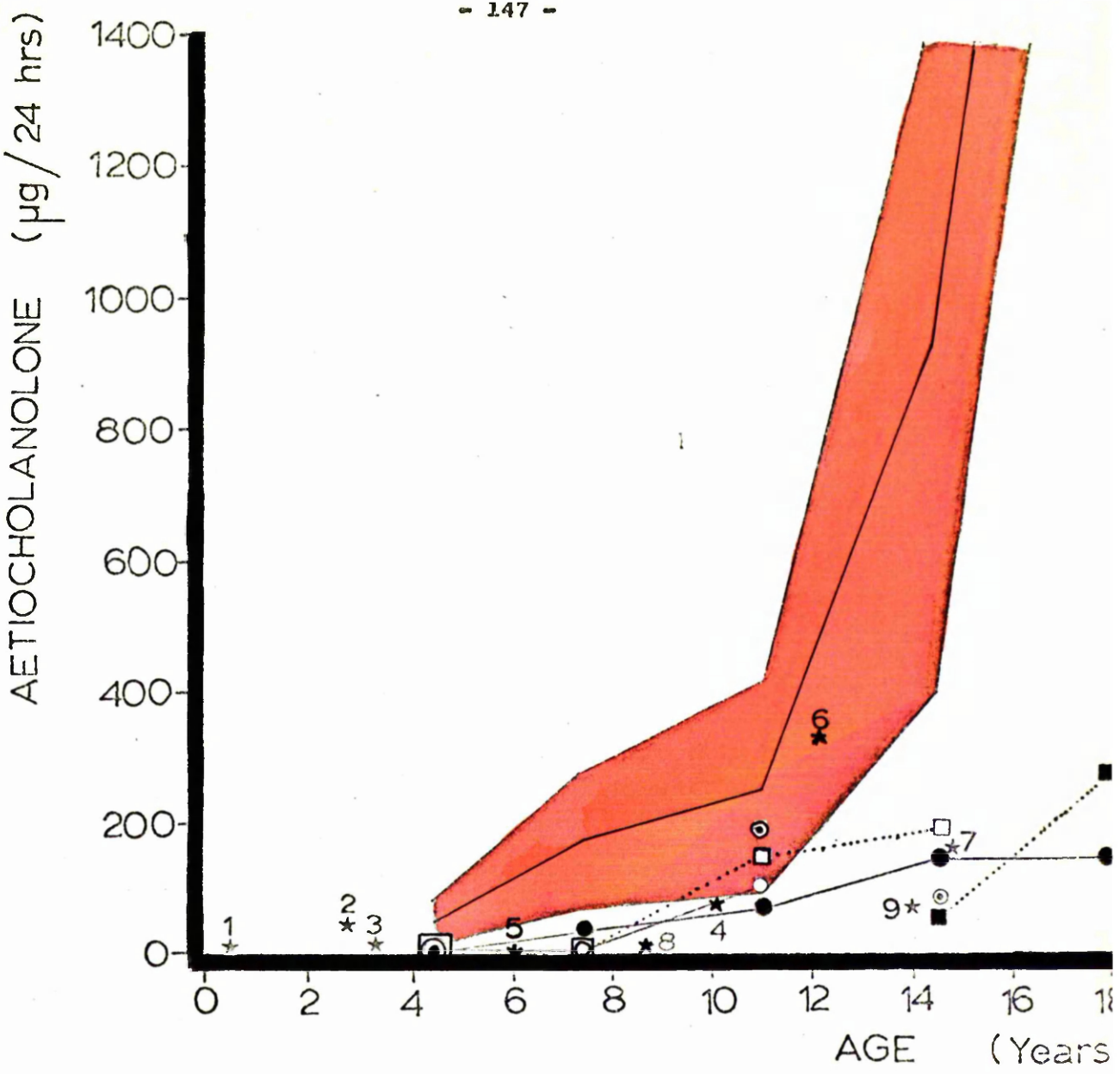


Figure 3.6 The mean basal urinary aetiocholanolone excretion by patients grouped by age, height and pubertal status. The shaded area represents the mean ± 1 SD of daily aetiocholanolone excretion by normal subjects as shown in Table 3.XI.

KEY:
- Patients with short stature
- Patients with bilateral undescended testes
- Patients with unilateral undescended testes
- Patients with hypogonadism (microgenitalia)
- Patients with delayed puberty
- ★ Patients with abnormal karyotype (identified by protocol number)

more rapid increase in aetiocholanolone excretion, whilst at puberty there is a very rapid rise in the aetiocholanolone excretion rate which persists during adolescence until adult levels are attained. Likewise, as the adrenal cortex is the main site of production of aetiocholanolone before puberty, the initial prepubertal rise in the urinary aetiocholanolone excretion probably represents the phenomenon of 'adrenarche', whilst the rapid pubertal rise is probably correlated to the rise in testicular secretion of precursors of aetiocholanolone.

It will be noted from Figure 3.6 that the basal urinary excretion of aetiocholanolone in patients with short stature is very low and continues to rise at the prepubertal rate, there being neither adrenal nor testicular correlated rise. The basal urinary excretion of aetiocholanolone in these patients was significantly below normal ($0.00005 < p < 0.02$) at all age groups.

The basal urinary excretion of aetiocholanolone in patients with bilateral undescended testes is likewise subnormal. Below 9 years of age the urinary excretion of aetiocholanolone in these patients is significantly below normal ($0.0001 < p < 0.02$) whilst in the older age group (9-13 years) the urinary excretion of aetiocholanolone remains significantly below normal ($0.01 < p < 0.02$) despite a significant ($p = 0.02$) adrenal correlated rise. No difference is found in urinary aetiocholanolone excretion between a patient with bilateral undescended testes (protocol 26, Table 3.X) and his two brothers following successful bilateral orchidopexy (protocols 27 and 28, Table 3.X) and presumably testicular hypofunction persists.

The basal urinary excretion of aetiocholanolone in young patients with unilateral undescended testes is much higher, being in the normal range. However, absence of the normal pubertal rise causes the levels of aetiocholanolone excretion in these patients to be significantly below normal ($0.001 < p < 0.002$) in older patients with unilateral undescended testes.

The basal urinary excretion of aetiocholanolone in patients with hypogonadism (small external genitalia) is very low. Below 9 years of age

the urinary excretion of aetiocholanolone in these patients is significantly below normal ($0.00005 < p < 0.05$) whilst in the older age groups (9-16 years) the urinary excretion of aetiocholanolone again remains significantly below normal ($0.02 < p < 0.05$) despite a very significant ($0.001 < p < 0.002$) adrenal correlated rise.

The basal urinary excretion of aetiocholanolone in a group of patients with delayed puberty (aged 13-16 years) was highly significantly below the normal range ($0.0001 < p < 0.0002$). It is again of possible clinical significance that this group of patients has neither adrenal nor pubertal correlated rises, whilst an older group of patients with delayed puberty had an adrenal but not a testicular correlated rise. The urinary aetiocholanolone excretion in this group of older patients with delayed puberty was still significantly subnormal ($0.02 < p < 0.05$).

Two patients with the Prader-Willi syndrome (protocols 58 and 72) have daily urinary aetiocholanolone levels significantly below normal ($0.05 < p < 0.10$) whilst an older patient with this syndrome (protocol 32) has a low normal level. One patient with the Laurence-Moon-Biedl syndrome with bilateral undescended testes has a basal urinary aetiocholanolone level equivalent to that found in patients with undescended testes.

Four patients with the testicular feminization syndrome (protocols 1-4) all have urinary aetiocholanolone excretions within the normal range. The basal level of aetiocholanolone excretion in two patients with Klinefelter's syndrome (protocols 8 and 9) are significantly below normal ($0.02 < p < 0.05$) and in agreement with the results of Johnsen (1956). Basal urinary aetiocholanolone excretion in two patients with the XO/XY syndrome is very variable. One patient (protocol 6) has a normal daily excretion level whilst the other (protocol 7) has a just significantly subnormal excretion ($0.05 < p < 0.10$). The daily urinary aetiocholanolone excretion in a patient with the XX/XY syndrome is significantly subnormal.

The urinary excretion of aetiocholanolone was significantly correlated with age ($r = 0.685$), bone age ($r = 0.693$) and pubertal stage ($r = 0.613$).

Again, the patients investigated were grouped according to height and pubertal status and the mean stimulation index calculated for each group. The results found for patients with 'near-normal' phenotype were again used for comparison. The results are shown in Table 3.XIII.

It will be noted from Table 3.XIII that the daily excretion of aetiocholanolone by normal patients here is approximately increased 1.7 fold following HCG stimulation. Although the stimulation index of urinary aetiocholanolone excretion is not significantly different from that of 'near-normal' patients in each of the phenotypes studied, it will be noticed from Table 3.XIII that the levels found closely resemble those found for the urinary androsterone excretion. Thus patients with Klinefelter's syndrome and patients with short stature (height percentile < 3) have, again, a very good stimulation of urinary aetiocholanolone excretion following HCG, whilst patients with the XO/XY syndrome have a poor increment in aetiocholanolone following HCG administration.

Dehydroepiandrosterone

The patients investigated were grouped according to age, height and pubertal status, as before. The daily basal urinary excretion of dehydroepiandrosterone (column G, Table 3.X) for each group was plotted against the mean age of the group. The graph is shown in Figure 3.7.

It will be noted that the shaded area of the graph represents the range (mean $\pm$ 1 SD) of daily urinary dehydroepiandrosterone (DHA) excretion calculated from the combined results of Vestergaard (1965), Paulsen *et al.* (1966) and Tanner and Gupta (1968), as shown in Table 3.XI. This shaded area therefore represents the normal DHA excretion, during childhood, puberty and adolescence. It will be noted that although the urinary excretion of DHA is much lower than that for either androsterone

TABLE 3.XIII EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON AETIOCHOLANOLONE EXCRETION BY DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)
'Near-normal' phenotype (n = 7)	1.69 ± 0.26
Short stature (n = 31)	3.07 ± 0.71
Bilateral undescended testes (n = 16)	2.41 ± 0.87
Delayed puberty (n = 6)	2.38 ± 0.61
Unilateral undescended testes (n = 6)	2.31 ± 0.40
Hypogonadism (n = 24)	2.31 ± 0.46
Testicular feminization syndrome (n = 4)	2.09 ± 0.59
XO/XY syndrome (n = 2)	1.59 ± 0.42
Prader-Willi syndrome (n = 3)	1.47 ± 0.63
Klinefelter's syndrome (n = 2)	7.35 ± 3.50
XX/XY syndrome (n = 1)	12.00

* Stimulation Index = $\frac{\text{Urinary aetiocholanolone excretion (3rd day)}}{\text{Basal urinary aetiocholanolone excretion}}$

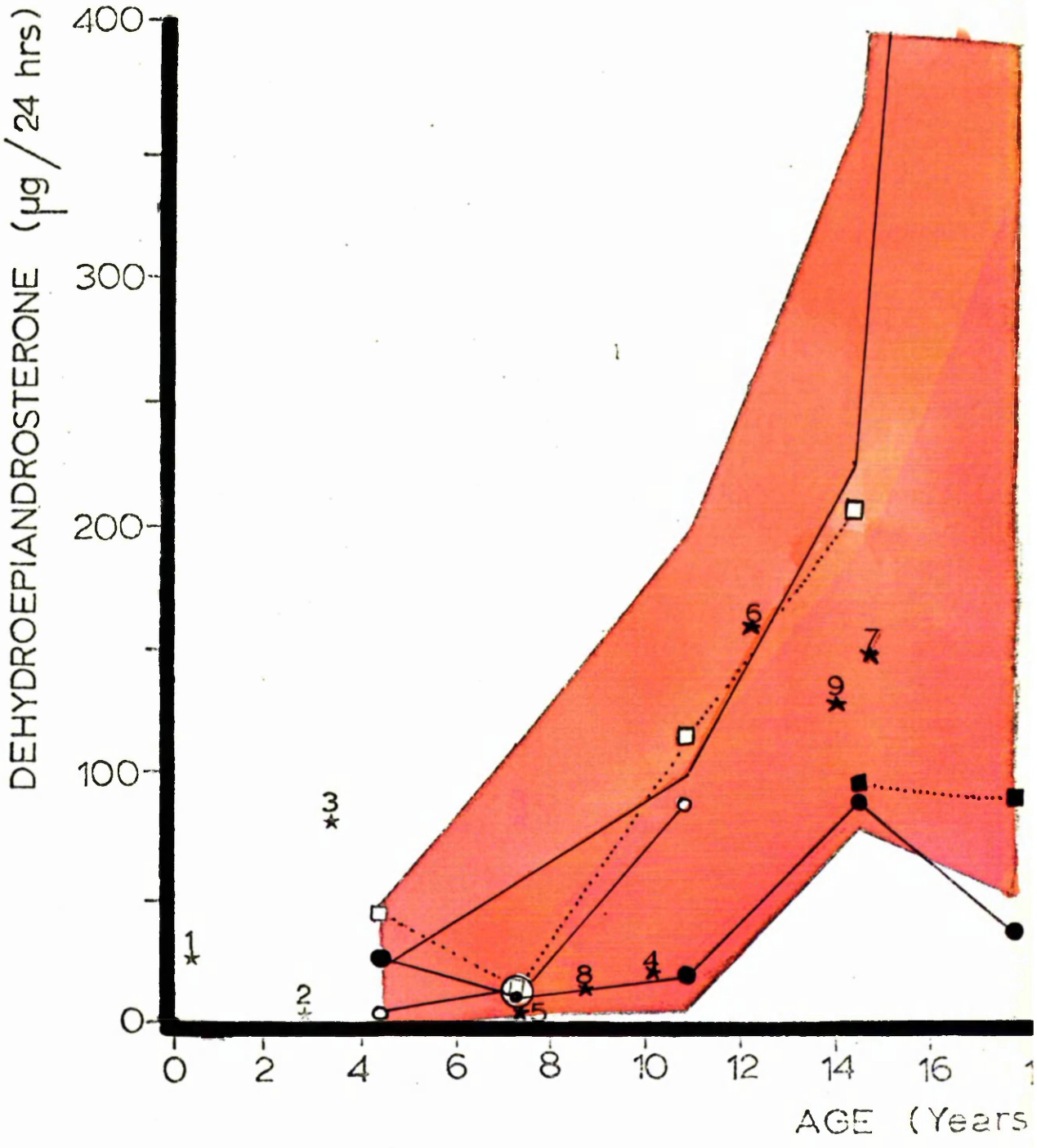


Figure 3.7 The mean basal urinary dehydroepiandrosterone excretion by patients grouped by age, height and pubertal status. The shaded area represents the mean ± 1 SD of daily dehydroepiandrosterone excretion by normal subjects as shown in Table 3.XI.

KEY:

- ◌ Patients with short stature (height percentile <3).
- ○ Patients with bilateral undescended testes.
- □ Patients with hypogonadism (microgenitalia).
- ◻ Patients with delayed puberty.
- ✱ Patients with abnormal karyotype (identified by protocol number).

or aetiocholanolone, the general shape of the curve is very similar. Again, there is a low, increasing daily DHA excretion during early childhood and a more rapid rise in the daily DHA excretion between 10 years and puberty. At puberty there is a very rapid increase which continues during adolescence to reach adult levels. It is probable that the initial prepubertal rise in urinary DHA excretion is correlated with increased adrenal production of DHAS whilst the pubertal rise is probably related to increased testicular activity.

It will be noted from Figure 3.7 that the basal urinary excretion of DHA in patients of short stature is very low, closely corresponding to the lower extremes of the normal range. Levels for these patients below 9 years of age are not significantly below the normal levels of DHA excretion. However, the delay in the adrenal correlated rise causes the urinary excretion of DHA to be highly significantly below normal ($0.0005 < p < 0.001$) in patients with short stature aged 9-13 years, whilst in patients in the pubertal and adolescent age groups the urinary excretion of DHA remained significantly below normal ($0.005 < p < 0.02$).

The basal daily excretion of DHA in patients with bilateral undescended testes is likewise very low during early childhood. The basal excretion of DHA between 3 and 6 years of age is significantly below normal ($0.0002 < p < 0.0005$), whilst in the next age group (6-9 years) it is just not significantly below normal ($0.05 < p < 0.10$). Above 9 years of age the daily excretion of DHA is in the normal range in these patients and there appears to be a normal adrenal correlated rise in the excretion. Again, no difference was noted in the daily DHA excretion of a patient with bilateral undescended testes (protocol 26) and his two brothers following successful bilateral orchidopexy (protocols 27 and 28).

However, the basal daily excretion of DHA in patients with unilateral undescended testes in the prepubertal age group (9-13 years) is much higher,

being within the normal range, but absence of the normal pubertal rise causes the levels of DHA excretion in older patients (13-16 years) to be significantly below normal ($0.01 < p < 0.02$).

The basal urinary excretion of DHA in patients with hypogonadism (small penis, small scrotum and microtestes) is within the normal range and increases normally with age. Similarly the basal urinary excretion of DHA in patients with delayed puberty, although at lower limits of normal, are not significantly different from normal ($0.1 < p < 0.2$).

The basal daily excretion of DHA in three patients with the Prader-Willi syndrome (protocols 32, 58 and 72) is within the low normal range and appears to increase normally with age. One patient with the Laurence-Moon-Biedl syndrome with bilateral undescended testes has a basal daily DHA excretion equivalent to that found in patients with undescended testes.

Four patients with the testicular feminization syndrome (protocols 1-4) all have a normal basal daily DHA excretion. The basal daily excretion in two patients with Klinefelter's syndrome is just within the low normal range. Similarly, the basal DHA excretion in two patients with the XO/XY syndrome is within the low normal range. The corresponding value in a patient with the XX/XY syndrome is just significantly below normal ($0.05 < p < 0.10$).

Many authors have disputed the presence of DHA in the urine of prepubertal children. Paulsen *et al.* (1966) could not detect DHA in the urine of any child below 8 years. Similarly, Beas *et al.* (1962) found no DHA in the urine of 5 boys aged 5 to 7 years. In this investigation urinary DHA was not detected in 7 patients and very low levels (< 10 µg per 24 hours) were found in a further 11 cases. It is interesting that 6 out of the 7 patients with undetectable urinary DHA have undescended testes, and 6 out of the 11 patients with a very low urinary DHA excretion also have undescended testes. This is suggestive of a deficiency of 17,20-desmolase in patients with undescended testes.

The urinary excretion of DHA was significantly correlated with age ($r = 0.394$), bone age ($r = 0.456$) and pubertal stage ($r = 0.462$).

Again the stimulation index was calculated in respect of DHA excretion for each patient. The patients investigated were grouped according to height and pubertal status and the mean stimulation index calculated for each group. Patients investigated with 'near-normal' phenotype were used for comparison. The results are shown in Table 3.XIV.

Here it will be noted that the daily excretion of DHA by normal patients in this investigation is approximately increased 1.8 fold following HCG stimulation. Note, too, that the stimulation index for the daily DHA excretion is very variable in patients with bilateral undescended testes and in patients with delayed puberty. Although the stimulation index of urinary DHA excretion is not significantly different from that of 'near-normal' patients in each of the phenotypes studied, it will be noticed from Table 3.XIV that the levels found closely resemble (with the exception of those for patients with bilateral undescended testes and patients with delayed puberty) those found for the rise in urinary aetiocholanolone excretion following HCG (Table 3.XIII, page 151).

Ratio of Androsterone to Aetiocholanolone

The principal end-products of androgen metabolism in man are the stereoisomers, androsterone and aetiocholanolone. They are formed in approximately equal quantities from the metabolism of the three main androgens, testosterone, androstenedione and dehydroepiandrosterone, and thus the ratio of urinary androsterone to urinary aetiocholanolone (A/Ae ratio) in normal subjects is approximately unity. (Baulieu and Mauvais-Jarvis, 1964; Johnsen, 1968).

Wilson and Schenker (1964) have shown that the A/Ae ratio is lowered by large doses of corticosteroids whilst Bradlow *et al.* (1956) found that the A/Ae ratio is dependent on the level of thyroid hormone. Johnsen (1968)

TABLE 3.XIV EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON DEHYDROEPIANDROSTERONE EXCRETION BY DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)[1]
'Near-normal' phenotype (n = 7)	1.78 ± 0.50
Bilateral undescended testes (n = 16)	6.79 ± 4.11
Delayed puberty (n = 6)	6.02 ± 4.62
Klinefelter's syndrome (n = 2)	3.98 ± 1.38
Short stature (n = 31)	3.09 ± 1.03
Testicular feminization syndrome (n = 4)	2.51 ± 0.91
Hypogonadism (n = 24)	1.45 ± 0.30
Unilateral undescended testes (n = 6)	1.39 ± 0.40
Prader-Willi syndrome (n = 2)	0.87 ± 0.01
XO/XY syndrome (n = 2)	0.83 ± 0.24
XX/XY syndrome (n = 1)	3.00

* Stimulation Index = $\frac{\text{Urinary DHA excretion (3rd day)}}{\text{Basal DHA excretion}}$

has shown that a raised A/Ae ratio, which may indicate hypothalamic disorders, is caused by a change in the metabolism of the principal androgens and not by abnormal steroid production.

The ratios of the daily urinary androsterone to the daily urinary aetiocholanolone excretion, calculated from the basal excretion and from the excretion on the third day of HCG stimulation, are shown in columns I and J respectively of Table 3.X. These ratios are compared to the normal A/Ae ratios found by Johnsen (1968).

It will be noted from Table 3.X that the basal A/Ae ratios found in many of the patients investigated are above the normal range and many of these elevated ratios are not suppressed by stimulation of testosterone synthesis by the administered HCG.

In order to discover if the A/Ae ratio is related to the phenotype of the patients investigated, the patients were grouped according to height and pubertal status and the mean A/Ae ratio calculated for each group. These results are shown in Table 3.XV.

It will be noted from this table that patients with 'near-normal' phenotype in this investigation, having normal daily basal and post-stimulation excretion of both androsterone and aetiocholanolone, have a mean A/Ae ratio within the normal range. This ratio is not significantly different from that found in the published normal cases of Johnsen (1968) and it does not alter significantly following stimulation with HCG. The ratios in the other phenotypes are thus compared to that found in these 'near-normal' patients.

Patients with hypogonadism (small penis, small scrotum and micro-testes), who have very low basal daily excretion levels of both androsterone and aetiocholanolone, have an elevated A/Ae ratio. This ratio is significantly ($0.01 < p < 0.02$) above that found in the 'near-normal' patients. As the stimulation of androsterone and aetiocholanolone

TABLE 3.XV RATIO OF URINARY ANDROSTERONE TO URINARY ABTIOCHOLANOLONE IN VARIOUS PHENOTYPES

Phenotype and Number	Mean A/Ae ratio (± 1 standard error)	
	BASAL	DAY 3
Normals (Johnsen, 1968) (n = 112)	1.04 (range 0.51-2.10)	-
'Near-normal' phenotype (n = 7)	1.79 ± 0.30	1.74 ± 0.29
Hypogonadism (n = 24)	6.06 ± 1.59*	3.29 ± 0.57*
Klinefelter's syndrome (n = 2)	5.63 ± 1.80	2.94 ± 0.11*
Testicular feminization syndrome (n = 4)	3.83 ± 1.51	2.28 ± 0.54
Bilateral undescended testes (n = 16)	2.92 ± 0.95	2.89 ± 0.72
Short stature (n = 29)	2.15 ± 0.34	2.30 ± 0.37
XO/XY syndrome (n = 2)	2.08 ± 0.65	2.07 ± 1.01
Delayed puberty (n = 6)	2.01 ± 0.34	1.70 ± 0.27
Unilateral undescended testes (n = 5)	1.57 ± 0.20	1.69 ± 0.18

* Significantly different from 'near-normal' phenotype

excretion following HCG is normal in these patients, it is possible that the defect may be of hypothalamic origin or, alternatively, caused by abnormal androgen metabolism.

Two patients with Klinefelter's syndrome (protocols 8 and 9), who have a normal daily excretion of androsterone but a decreased excretion of aetiocholanolone, have an elevated A/Ae ratio. This ratio is just not significantly above that found in the 'near-normal' patients. The rise of androsterone and aetiocholanolone excretions following HCG is also raised in these patients. This is consistent with abnormal androgen metabolism.

Four patients with the testicular feminization syndrome, with basal androsterone and aetiocholanolone excretions within the normal range, have a raised A/Ae ratio although this is not significantly above that of 'near-normal' patients. As the response of androsterone and aetiocholanolone excretion to HCG administration was normal in these patients, normal androgen metabolism must be postulated. This is consistent with the defect being an end-organ insensitivity to androgen in such patients.

Patients with bilateral undescended testes have an A/Ae ratio which, although raised, is not significantly different ($0.2 < p < 0.5$) from that of 'near-normal' patients. These patients have a good increase in androsterone and aetiocholanolone excretion following HCG from very low basal levels. The defect in these patients is thus probably related to partial gonadotrophin failure.

In patients with short stature, having very low basal daily excretions of both androsterone and aetiocholanolone, the A/Ae ratio is not significantly different from that found in the 'near-normal' patients. It is therefore postulated that the extremely low excretion of androgen metabolites in these patients is not caused by abnormal metabolism but rather by reduced synthesis of androgens.

In patients with delayed puberty, who have similarly very low basal daily excretion of androsterone and aetiocholanolone, the A/Ae ratio is similarly not significantly different from that found in patients of 'near-normal' phenotype. Again it is probable that the extremely low daily excretion of the androgen metabolites in these patients reflects a low synthesis of androgens and it is this low androgen production which induces the delayed puberty.

The patient with the XX/XY genotype (protocol 40), who has extremely low basal levels of androsterone and aetiocholanolone excretion, also has a greatly elevated A/Ae ratio. The A/Ae ratio of 11.00 is statistically significant when compared with that of 'near-normal' patients, and this is consistent with an abnormal androgen production that would be expected in subjects with ovotestes.

Two patients with the XO/XY syndrome have A/Ae ratios not significantly different from that found in 'near-normal' patients. As the basal daily levels of androsterone and aetiocholanolone excretion in these patients are within the normal range and increase normally with HCG administration, it would appear that the metabolism of androgens in this syndrome is identical with that of normal males.

Two patients with precocious puberty (protocols 77 and 78) have greatly elevated A/Ae ratios. The mean A/Ae ratio of 8.42 is significantly above that of 'near-normal' patients. This elevated A/Ae ratio may be related either to an over-production of androgens or to an abnormal androgen metabolism.

The A/Ae ratios in three patients with the Prader-Willi syndrome were very variable. Although the mean A/Ae ratio of 10.29 is grossly elevated this is not significantly above that of 'near-normal' patients owing to large variation. However, it is interesting that Johnsen (1968) described elevated A/Ae ratios in patients with similar phenotypes (ie hypogonadism with obesity) and related this to a hypothalamic defect.

When the A/Ae ratios on the third day of HCG are compared to the A/Ae ratios of the basal day (Table 3.XV) no significant change in androgen metabolism is noted. However, there is a trend towards lower (normal) results following HCG. Note, too, that in a patient with the XX/XY genotype and ovotestes the grossly elevated A/Ae ratio of the basal day is reduced to the normal level following HCG administration. This is consistent with an abnormal basal androgen metabolism which is normalised, although not greatly elevated, by stimulation of the Leydig cells with gonadotrophin. Thus it may be that the gonadotrophin (HCG) administered has other extra-testicular effects which influence metabolic pathways and even tissue sensitivity to metabolically active androgens.

Epiandrosterone

Very few normal levels for the daily urinary excretion of epiandrosterone (3β-hydroxy-5α-androstan-17-one) in children and adolescents have been reported in the literature. Paulsen et al. (1966) found undetectable urinary levels in children before 9 years of age and reported low levels of urinary epiandrosterone in only three male children in early puberty. Uozumi et al. (1969) confirmed the negligible urinary epiandrosterone excretion in young children and reported mean levels of daily urinary epiandrosterone excretion in early puberty, adolescence and throughout adulthood in males. In females they reported negligible epiandrosterone excretion until adulthood and even then the levels were below those found in males. Feher (1967a) reported levels of urinary epiandrosterone in normal adults and in patients with various endocrine disorders.

The basal daily urinary epiandrosterone excretion rates found in this investigation (Table 3.XVI) were initially compared to the normal values reported by Uozumi et al. (1969). The basal urinary epiandrosterone excretion (column C, Table 3.XVI) was plotted against patient age. This graph is shown in Figure 3.8. Here it will be noted that the shaded area

TABLE 3.XVI DAILY URINARY EXCRETION OF EPIANDROSTERONE AND THE EFFECT OF ADMINISTERED HCG

Protocol	Age (Years)	Epiandrosterone Excretion (µg/24 hrs)		Protocol	Age (Years)	Epiandrosterone Excretion (µg/24 hrs)	
		Basal	Day 3			Basal	Day 3
A	B	C	D	A	B	C	D
1	0.4	129	356	41	4.2	1	6
2	2.8	78	1,163	42	13.0	2	13
3	3.3	120	124	43	14.0	15	4
4	10.1	2	6	44	14.0	5	5
5	6.0	1	1	45	16.0	49	11
6	12.3	8	19	46	16.0	9	76
7	14.8	657	640	47	16.0	12	16
8	8.7	45	10	48	16.0	17	37
9	14.0	238	3,289	49	16.1	4	12
10	8.1	42	47	50	17.1	5	6
11	8.5	10	20	51	3.8	81	4
13	11.0	11	10	52	6.8	64	57
14	11.5	73	2	53	10.9	742	2,078
15	12.2	17	6	54	11.0	105	611
16	14.3	15	10	55	12.6	53	7
17	15.0	46	30	56	13.7	247	533
18	5.4	2	7	57	13.9	261	316
19	6.0	10	8	58	4.5	572	415
20	8.3	47	164	59	5.4	33	46
21	10.1	15	17	60	9.5	57	6
22	10.3	16	42	61	9.6	136	161
23	11.8	196	202	62	10.0	194	ND*
24	15.0	100	9	63	10.9	486	497
25	15.3	382	ND	64	14.4	214	158
26	4.1	4	1	65	15.0	36	42
27	6.0	50	4	66	15.6	24	13
28	7.3	19	195	67	20.0	14	36
29	8.2	1	7	68	26.0	24	23
30	10.8	2	4	69	12.1	353	1,388
31	10.8	12	3	70	19.9	291	276
32	11.0	8	8	71	6.3	83	5
33	11.2	4	3	72	8.0	6	11
34	11.9	1	26	73	15.5	40	17
35	13.5	7	1	74	8.3	1	2
36	14.8	4	5	75	7.0	23	17
37	15.0	15	39	76	9.0	119	20
38	16.1	16	6	79	17.2	10	6
39	19.0	6	6	80	14.0	34	89
40	4.1	4	14				

* ND = Not Detectable

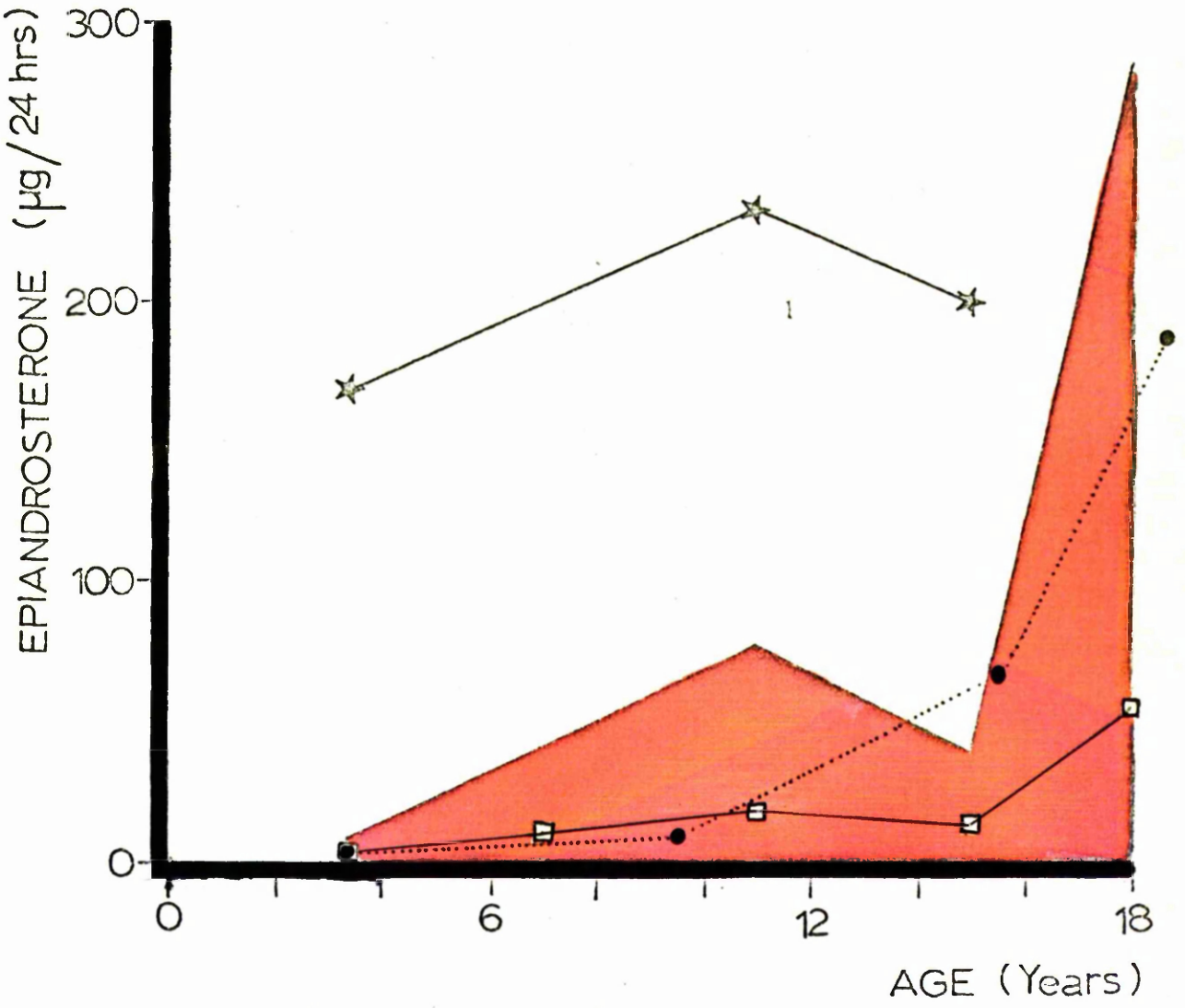


Figure 3.8 The mean basal urinary epiandrosterone excretion by patients grouped by age and pubertal status. The shaded area represents the mean ± 2 SD of normal urinary excretion of epiandrosterone found in this investigation.

KEY:

□ Mean normal excretion found in this investigation

○ Mean normal excretion (Uozumi et al., 1969)

✳ Mean excretion in patients with raised urinary excretion of epiandrosterone

of the graph represents the range ($\pm$ 2 SD) of normal daily urinary epiandrosterone excretion found in this investigation.

There is an extremely low daily excretion of urinary epiandrosterone throughout the prepubertal, pubertal and adolescent age groups studied. Note, too, the absence of any significant rise in daily epiandrosterone excretion as occurred with the other 11-deoxy-17-oxosteroids during the late prepubertal or pubertal periods. As there is no sudden rise at puberty it is improbable that the main precursor of epiandrosterone is testosterone, as reported by Fukushima et al. (1954), since normally there is a marked rise in testosterone at puberty.

It will also be noted further from Figure 3.8 that this normal range of daily urinary epiandrosterone excretion closely resembles that found in the normal cases of Uozumi et al. (1969). However, the daily basal urinary epiandrosterone excretion in other patients in my series was greatly elevated. No correlation was found in these patients between the phenotype and elevated epiandrosterone excretion, although it is interesting that the majority of these patients had undescended testes. It is probable that the elevated daily urinary epiandrosterone excretion found in these patients reflects abnormal androgen metabolism. The precursors of epiandrosterone were therefore studied to investigate the possibility of abnormal androgen metabolism in these patients.

Dorfman and Shipley (1956) reported three theoretical pathways between testosterone and epiandrosterone. The normally accepted pathway of catabolism proceeds through Δ^4-androstenedione and 5α-androstanedione and favours the formation of androsterone (the 3α-metabolite). Epiandrosterone synthesis and its urinary excretion are therefore normally low. Metabolism of testosterone in androgen-dependent tissues may, however, take place through 5α-dihydrotestosterone and 5α-androstane-3β,17β-diol to yield epiandrosterone, whilst the third possible metabolic pathway proceeds through Δ^5-androstene-3β,17β-diol and dehydroepiandrosterone.

Raised urinary epiandrosterone excretion can therefore be caused by a relative change in the activities of 3α- or 3β-dehydrogenase in favour of 3β-enzyme, or possibly by tissue metabolism of another androgen (probably DHA) in the event of either inadequate levels of testosterone or tissue insensitivity.

In order to evaluate further the state of the 3α- and 3β-dehydrogenases, the basal daily urinary epiandrosterone excretion in my series was plotted against the basal urinary excretion of androsterone for each patient. This graph is shown in Figure 3.9.

There the shaded area of the graph represents the normal ratio of urinary excretion of androsterone to the urinary excretion of epiandrosterone (A/EpiA ratio). All patients in this investigation with normal basal levels of urinary epiandrosterone excretion are within this range, in agreement with the mean A/EpiA ratio of 12.25 calculated from the data of Uozumi et al. (1969). The lower limit of this normal range was taken as an A/EpiA ratio greater than 5, after scrutiny of the normal range of urinary epiandrosterone excretion, as shown in Figure 3.8.

All patients with elevated levels of epiandrosterone excretion have a low A/EpiA ratio and are therefore below the normal range. These patients were divided into two groups for further evaluation, patients with a raised daily epiandrosterone excretion and with an A/EpiA ratio greater than 1, and those with a raised urinary epiandrosterone excretion and with an A/EpiA ratio less than 1.

In each of these groups of patients the basal daily urinary epiandrosterone excretion was compared to that of other steroids. Spearman correlation coefficients were calculated between the excretion rates of these steroids in each group of patients. These are shown, where significant, in Table 3.XVII.

There it will be noted that for patients with a normal basal urinary epiandrosterone excretion, the excretion of epiandrosterone is

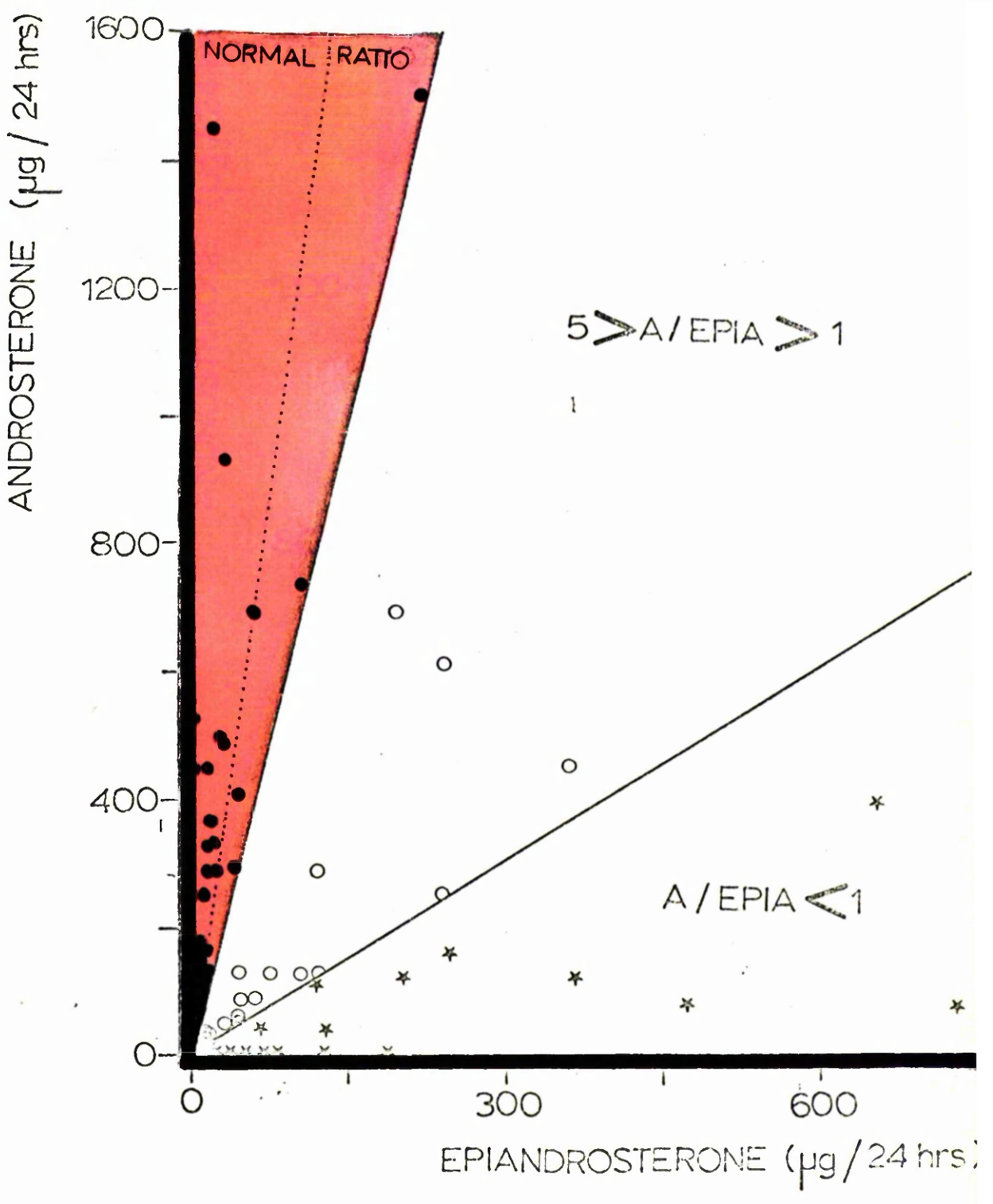


Figure 3.9 The basal urinary excretion of epiandrosterone by individual patients compared to the basal urinary excretion of androsterone. The shaded area represents the mean ratio (Uozumi et al., 1969) and normal range found in 'near-normal' patients in this investigation.

KEY:
- ⊙ Patients with normal A/EpiA ratio
- ○ Patients with elevated A/EpiA ratio (Epia < A)
- ✱ Patients with elevated A/EpiA ratio (Epia > A)

TABLE 3.XVII SIGNIFICANT SPEARMAN CORRELATION COEFFICIENTS OF BASAL URINARY EPIANDROSTERONE EXCRETION WITH BASAL URINARY EXCRETION OF OTHER STEROIDS

Steroid*	Patients with Normal Epiandrosterone Excretion	Patients with Elevated Epiandrosterone Excretion	
		EpiA < A	EpiA > A
A	0.690	0.934	0.787
Ae	0.476	0.730	0.725
DHA	0.617	0.806	0.750
5α-Diol	0.480	-	-
5β-Diol	0.449	-	-
3β-Diol	0.328	0.452	-
PT	-	-	0.618

* Abbreviations used:

A	Androsterone
Ae	Aetiocholanolone
DHA	Dehydroepiandrosterone
5α-Diol	5α-Androstane-3α,17β-diol
5β-Diol	5β-Androstane-3α,17β-diol
3β-Diol	5α-Androstane-3β,17β-diol
PT	Plasma testosterone

significantly correlated with the urinary excretion of androsterone, aetiocholanolone, dehydroepiandrosterone and the three androstanediols. It is therefore possible that in these patients epiandrosterone production is dependent on the relative activities of Δ^4-5α- and Δ^4-5β-reductase enzymes reducing Δ^4-androstenedione, and on the relative activities of 3α- and 3β-dehydrogenase reducing 5α-androstanedione (ie the normal hepatic metabolism of androgens, Figure 1.2, page 15). DHA catabolism to epiandrosterone in these patients would then proceed through Δ^4-androstenedione, as a Δ^5-reductase, involved in direct metabolism of DHA to epiandrosterone, has not been identified. The androstanediols would of course arise from further metabolism of the 17-oxosteroids.

It is interesting that in these patients the urinary excretion of epiandrosterone was significantly correlated with the pubertal ($r = 0.358$) and gonadal ($r = 0.368$) status.

In patients with a raised basal urinary excretion of epiandrosterone but less than that for androsterone (A/EpiA ratio > 1) the excretion of urinary epiandrosterone is significantly correlated with the urinary excretion of androsterone, aetiocholanolone, dehydroepiandrosterone and 5α-androstane-3β,17β-diol.

Two possible explanations for the raised epiandrosterone excretion in these patients can be postulated. Firstly, the raised epiandrosterone excretion may arise from a change in the relative activities of the Δ^4-5α- and Δ^4-5β-reductases reducing androstenedione to give a preponderance of 5α-reduced metabolites. A change in the relative activities of the 3α- and 3β-dehydrogenases to reduce the formed 5α-androstanedione, giving a preponderance of 3β-reduced metabolites, would also be necessary to produce high urinary excretion of epiandrosterone (some six times normal levels) in combination with normal levels of urinary androsterone excretion. Under these conditions DHA catabolism to epiandrosterone would presumably take place through Δ^4-androstenedione.

Secondly, a direct metabolism of DHA to epiandrosterone, involving an unidentified Δ^5-reductase, may be postulated. Under these conditions androsterone could then arise from epiandrosterone, probably through 5α-androstanedione (the 3-oxo intermediate) as described by Baulieu et al. (1965). In the patients with elevated urinary epiandrosterone excretion it should be noted that the urinary excretion of androsterone, aetiocholanolone and DHA is not significantly different from normal excretion. This precludes a change in the relative activities of Δ^4-5α- and Δ^4-5β-reductases and 3α- and 3β-dehydrogenases and favours the second hypothesis of direct formation of epiandrosterone from DHA.

If this second hypothesis of direct metabolism from DHA is accepted, it can then be postulated that when general tissue metabolism cannot utilize testosterone, either because of inadequate testosterone synthesis or tissue insensitivity to testosterone, then DHA becomes the principal anabolic hormone (Hamilton, 1974a). DHA production then rises in such patients and the urinary excretion of epiandrosterone increases.

A very brief comment on patients of this group with raised urinary excretion of epiandrosterone may clarify these points. Of the 19 patients investigated in this group, 5 (26 per cent) were found to have primary testicular failure with low levels of testosterone. A further 6 patients (32 per cent) had very low levels of plasma testosterone, whilst 3 patients (16 per cent) presented with varying degrees of tissue insensitivity (ie near normal plasma levels of testosterone but with micropenis and microscrotum). One of the patients with elevated urinary epiandrosterone excretion had precocious puberty. The raised urinary epiandrosterone excretion in this patient is certainly not secondary to tissue insensitivity or testosterone insufficiency but probably due to treatment of the patient during the period of investigation with cyproterone acetate, a potent antiandrogen. This drug has recently been reported to inhibit 3β-hydroxy-Δ^5-steroid dehydrogenase (Goldman, 1972).

In these patients raised urinary epiandrosterone excretion may indicate a somatic defect, secondary either to tissue insensitivity to testosterone, or to testicular insufficiency (ie testosterone lack). In these circumstances, the soma cannot utilize testosterone and growth and development are dependent on the weaker androgen, DHA.

In patients with a raised basal urinary excretion of epiandrosterone above that of androsterone (A/EpiA ratio < 1) the excretion of urinary epiandrosterone is significantly correlated with the urinary excretion of androsterone and aetiocholanolone. It is therefore possible that in these patients epiandrosterone production is dependent on the relative activities of Δ^4-5α- and Δ^4-5β-reductase enzymes reducing Δ^4-androstenedione and on the relative activities of 3α- and 3β-dehydrogenases reducing the formed 5α-androstanedione. Furthermore, as the urinary excretions of androsterone and aetiocholanolone are significantly below normal ($0.001 < p < 0.01$) in these patients, it is postulated that the raised urinary epiandrosterone excretion arises from a change in the relative activities of these two enzyme systems to give a preponderance of 5α- and 5α,3β-reduced products respectively. This hypothesis explains the raised urinary epiandrosterone excretion in association with subnormal urinary excretion of the other 11-deoxy-17-oxosteroids as seen in these patients.

It is interesting that of the 18 patients investigated in this group, 12 (67 per cent) have undescended testes. Furthermore, in all these patients with undescended testes the urinary excretion of epiandrosterone exceeds that of the combined excretions of the other 11-deoxy-17-oxosteroids. It is therefore postulated that the raised urinary epiandrosterone excretion found in the patients with undescended testes may reflect abnormal steroid metabolism in the cryptorchid testes and this intrinsic metabolic defect may cause non-descent of the testes.

Of the remaining patients within this group, 3 patients were of short stature but with normal levels of human growth hormone, 2 patients have hypogonadism, whilst the remaining patient had previously been treated with cyclophosphamide for the nephrotic syndrome. Cyclophosphamide (1-bis-(2-chloroethyl)amino-1-oxo-2-azo-6-oxaphosphoridine), a heterocyclic mustard, is known to cause testicular tubular damage but is believed to have no effect on interstitial tissue (Kumar et al., 1972). These patients are distinguished from those with undescended testes by lower levels of urinary epiandrosterone excretion and they may therefore represent intermediate examples in severity of the enzyme defect postulated.

On stimulation with HCG, the daily urinary excretion of epiandrosterone was remarkably variable. Of the patients investigated, the response (column D, Table 3.XVI) was increased above the basal excretion in 41 per cent. No response occurred in 26 per cent, whilst for the remaining patients (33 per cent) there was a diminished excretion of urinary epiandrosterone below basal levels.

Although no general correlation was found between the response of epiandrosterone excretion after HCG stimulation to the phenotype of the patient, it should be noted that patients with gynaecomastia have extremely raised epiandrosterone excretion after HCG. As the basal urinary epiandrosterone excretion in these patients is significantly above normal, and is in association with low or normal levels of the other 11-deoxy-17-oxosteroids, it is postulated that in these patients there is a partial deficiency of 3β-hydroxysteroid dehydrogenase. This enzyme deficiency may, however, be limited to the testicular or gynaecomastic tissue only, as complete or partial deficiencies of adrenal 3β-hydroxysteroid dehydrogenase are characteristic of congenital adrenal hyperplasia with the salt-losing syndrome, ambiguous genitalia and often early death (Goldman et al., 1964; Janne et al., 1970). Recently, Parks

et al. (1971) reported a 13-year-old male child with this enzyme defect who had severe gynaecomastia.

11β-Hydroxyandrosterone

The daily urinary excretion of 11β-hydroxyandrosterone of the patients investigated is shown in Table 3.XVIII. The basal urinary excretion of 11β-hydroxyandrosterone (column C, Table 3.XVIII) was plotted against the age of the patient. This graph is shown in Figure 3.10.

It will be seen that the shaded area of the graph represents the range ($\pm$ 2 SD) of daily urinary 11β-hydroxyandrosterone excretion calculated from the combined results of Vestergaard (1965), Paulsen et al. (1966) and Tanner and Gupta (1968) for normal children and adolescents, as shown in Table 3.XIX. This shaded area therefore represents, for the present purposes, the normal range of daily 11β-hydroxyandrosterone excretion.

The shape of this curve is of interest. There is a low daily excretion of 11β-hydroxyandrosterone for patients less than 10 years. This then rises more rapidly before puberty. At puberty there is no rapid rise, as seen with androsterone excretion. Again, as the adrenal cortex is the main source of production of 11β-hydroxyandrosterone, the prepubertal rise in the daily urinary 11β-hydroxyandrosterone excretion probably represents the phenomenon of 'adrenarche' whilst the absence of a pubertal rise is consistent with the fact that all precursors of 11β-hydroxyandrosterone are adrenal and not testicular in origin.

The basal daily excretion of 11β-hydroxyandrosterone in patients of short stature (height percentile <3) is very low, although it increases with age normally. Between 6 and 9 years of age the basal daily excretion of 11β-hydroxyandrosterone is not significantly different from normal. In the late prepubertal age group, 9 to 13 years, the daily excretion of 11β-hydroxyandrosterone is significantly below the normal range ($0.005 < p < 0.01$) whilst in the pubertal and adolescent age groups

TABLE 3.XVIII DAILY URINARY EXCRETION OF 11β-HYDROXYANDROSTERONE AND 11β-HYDROXYAETIOCHOLANOLONE AND THE EFFECT OF ADMINISTERED HCG

Protocol	Age (Years)	11β-OH-A Excretion (µg/24 hrs)		11β-OH-Ae Excretion (µg/24 hrs)		Ratio $\frac{11\beta\text{-OH-A}^*}{11\beta\text{-OH-Ae}}$	
		Basal	Day 3	Basal	Day 3	Basal	Day 3
A	B	C	D	E	F	G	H
2	2.8	1,102	1,159	-	-	-	-
11	8.5	176	848	113	859	1.56	0.99
16	14.3	128	120	248	60	0.52	2.00
23	11.8	332	278	64	140	5.19	1.99
26	4.1	139	139	37	31	3.76	4.48
27	6.0	109	108	-	-	-	-
28	7.3	374	368	31	72	12.06	5.11
33	11.2	593	584	-	-	-	-
35	13.5	120	120	136	106	0.88	1.13
37	15.0	459	574	-	-	-	-
38	16.1	274	258	-	-	-	-
39	19.0	296	332	84	58	3.52	5.72
44	14.0	430	492	54	315	7.96	1.56
45	16.0	475	404	66	128	7.20	3.16
46	16.0	614	617	-	-	-	-
47	16.0	1,341	1,269	278	450	4.82	2.82
48	16.0	449	427	-	-	-	-
49	16.1	530	546	341	138	1.55	3.96
55	12.6	364	300	47	90	7.74	3.33
56	13.7	710	-	18	-	39.44	-
59	5.4	198	209	89	39	2.22	5.36
60	9.5	18	103	5	88	3.60	1.17
62	10.0	39	36	67	78	0.58	0.46
65	15.0	1,749	1,768	1,047	993	1.67	1.78
68	26.0	1,100	1,108	177	110	6.21	10.07
69	12.1	2,282	2,242	242	344	9.43	9.19
70	19.9	3,359	3,027	-	-	-	-
73	15.5	403	442	438	399	0.92	1.11
75	7.0	266	274	259	188	1.03	1.46
78	5.0	324	-	40	-	8.10	-

* Normal values Forsyth (1974)

Age group	11β-OH-A/11β-OH-Ae ratio
1-6	1.4
7-10	1.6
11-17	1.7
Adults	3.0

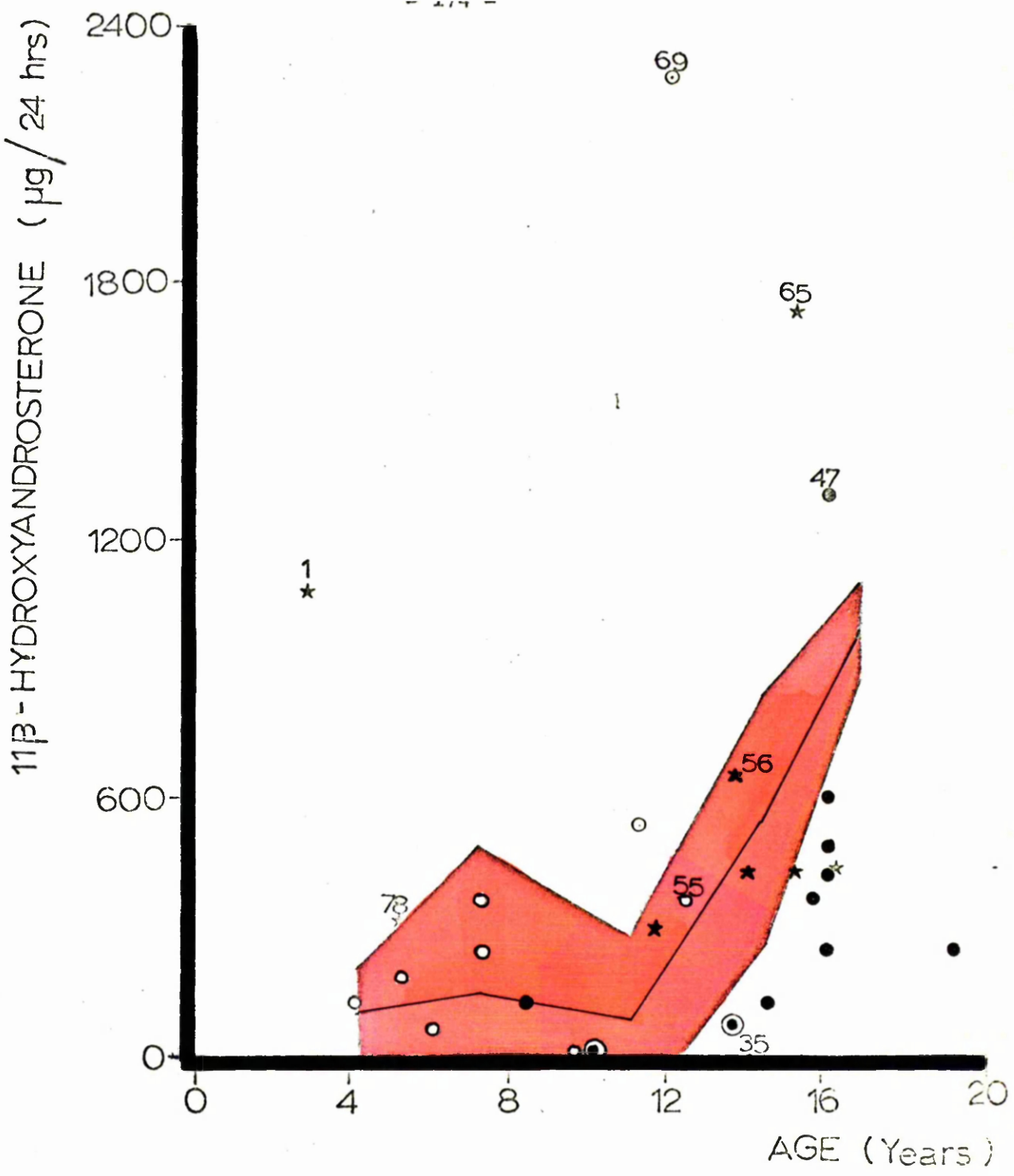


Figure 3.10 The basal urinary excretion of 11β-hydroxyandrosterone in patients grouped by age, height and pubertal status. The shaded area represents the mean ± 2 SD 11β-hydroxyandrosterone excretion by normal subjects as shown in Table 3.XIX. Numbers refer to protocols of patients mentioned in the text.

KEY: ○ Patients with undescended testes

⊙ Patients with short stature (height percentiles <3)

☆ Other patients

TABLE 3.XIX MEAN DAILY URINARY EXCRETION OF 11β-HYDROXYANDROSTERONE AND 11β-HYDROXYAETIOCHOLANOLONE IN NORMAL CHILDREN AND ADOLESCENTS*

Age Range (years)	11β-OH Androsterone (μg/24 hrs)	11β-OH Aetiocholanolone (μg/24 hrs)
3-6	103 ± 23 n = 4	48 ± 16 n = 8
6-9	129 ± 48 n = 13	117 ± 28 n = 16
9-13	78 ± 17 n = 36	114 ± 10 n = 41
13-16	553 ± 83 n = 3	338 ± 151 n = 4
16+	1000 ± 40 n = 2	910 ± 510 n = 2

* Mean ± 1 standard error calculated from the normal results of Vestergaard (1965), Paulsen et al. (1966) and Tanner and Gupta (1968)

this excretion, although rising, remains subnormal ($0.01 < p < 0.05$). As the urinary excretion of 11β-hydroxyandrosterone rises normally with age in these patients with short stature and, furthermore, is at a normal level when adjusted to a normal urinary creatinine excretion normal adrenal function must be concluded. The low urinary excretion of 11β-hydroxyandrosterone in such patients is probably related to the reduced adrenal gland size.

The basal daily excretion of 11β-hydroxyandrosterone in patients with bilateral undescended testes, although not significantly above normal, is generally within the high normal range of excretion. However, the basal urinary excretion of 11β-hydroxyandrosterone in the eldest patient with undescended testes (protocol 69, aged 12.1 years), at 2,282 µg per 24 hours, is well above the normal range of excretion (78 ± 17 µg per 24 hours).

The basal daily excretion of 11β-hydroxyandrosterone in patients with unilateral undescended testes is variable. In one such patient (protocol 55) the value is significantly raised ($0.002 < p < 0.005$) whilst in a further patient (protocol 35) the excretion of 11β-hydroxyandrosterone is significantly below normal ($0.005 < p < 0.01$).

In hypogonadal patients (small external genitalia) the basal excretion was within the normal range, whilst in one patient with the Laurence-Moon-Biedl syndrome (in which hypogonadism is one of the presenting features) the basal excretion of 11β-hydroxyandrosterone was within the high normal range and is consistent with such patients with bilateral undescended testes.

In a patient with precocious puberty (protocol 78) the basal excretion was significantly above ($0.02 < p < 0.05$) the normal range for chronological age. However, it is noteworthy that the excretion in this patient (324 µg per 24 hours) is at the mean level when plotted against the patient's advanced bone age.

The basal daily urinary excretion of 11β-hydroxyandrosterone in three patients with Cushing's syndrome was generally raised. One of these patients (protocol 37) had a normal excretion of 11β-hydroxyandrosterone, whilst in the two other patients with Cushing's syndrome this was significantly above normal ($0.01 < p < 0.02$). This is consistent with the elevated corticosteroid production in this syndrome and these are precursors of urinary 11β-hydroxyandrosterone.

The fact that the post-stimulation excretion of 11β-hydroxyandrosterone was not significantly different from the basal levels is consistent with the established concept that the precursors of 11β-hydroxyandrosterone are of adrenal and not testicular origin.

11β-Hydroxyaetiocholanolone

The basal daily excretion of 11β-hydroxyaetiocholanolone (column E, Table 3.XVIII) of the patients investigated was plotted against the age of the patient. This graph is shown in Figure 3.11.

It will be noted that the shaded area of the graph represents the range ($\pm$ 2 SD) of daily urinary 11β-hydroxyaetiocholanolone excretion calculated from the combined results of Vestergaard (1965), Paulsen et al. (1966) and Tanner and Gupta (1968), as shown in Table 3.XIX. This shaded area therefore represents, for the present purposes, the normal range of daily 11β-hydroxyaetiocholanolone excretion.

Again, there is a low daily excretion of 11β-hydroxyaetiocholanolone by patients less than 10 years of age. The level then rises more rapidly before puberty. Again, as the adrenal cortex is the main source of 11β-hydroxyaetiocholanolone, this rise probably represents the phenomenon of 'adrenarche'. The absence of a further pubertal rise is consistent with the fact that all precursors of 11β-hydroxyaetiocholanolone are of adrenal and not testicular origin.

All basal daily excretions of 11β-hydroxyaetiocholanolone of the patients investigated were within the limits of the normal range ($\pm$ 2 SD).

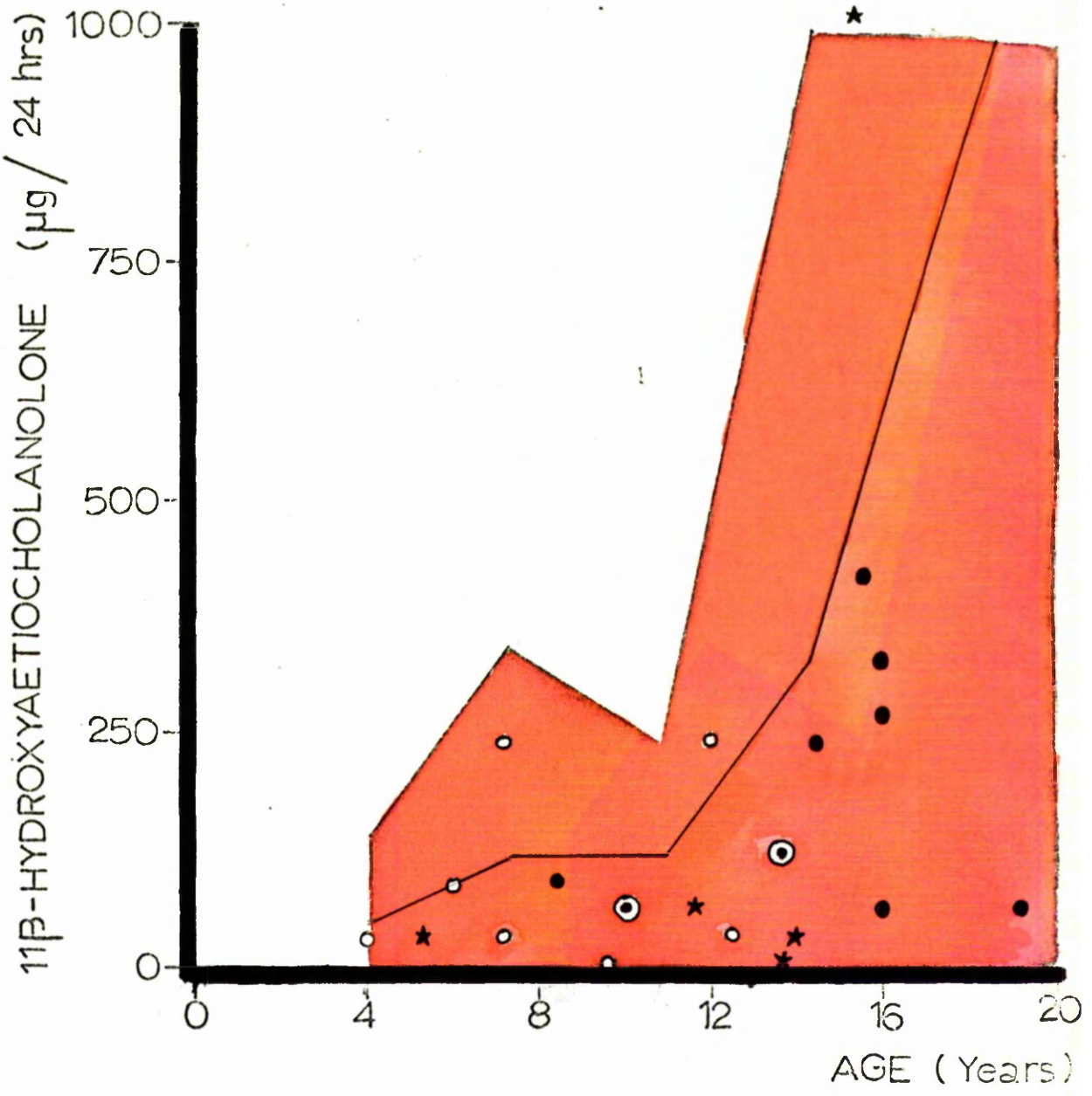


Figure 3.11 The basal urinary excretion of 11β-hydroxyaetiocholanolone in patients grouped by age, height and pubertal status. The shaded area represents the mean $\pm$ 2 SD 11β-hydroxyaetiocholanolone excretion by normal subjects as shown in Table 3.XIX.

KEY:
- ● Patients with short stature (height percentiles < 3)
- o Patients with undescended testes
- ✱ Other patients

The small number of determinations prevented statistical comparison of patients grouped by phenotype. However, as the normal range of excretion of 11β-hydroxyaetiocholanolone is so variable, it is probable that if more analyses had been carried out the results would not yield values significantly different from normal.

The basal daily excretion of 11β-hydroxyaetiocholanolone in patients of short stature is again, however, in the low normal range of excretion, as seen with 11β-hydroxyandrosterone studies. The corresponding values for patients with bilateral undescended testes is, however, much lower than that for the 5α-epimer. Normal excretion levels of 11β-hydroxyaetiocholanolone were found in four patients, whilst in a further two patients with this phenotype very low levels were found.

On stimulation with HCG, the daily urinary excretion of 11β-hydroxyaetiocholanolone was very variable. Although increased excretion of 11β-hydroxyaetiocholanolone was noted in nearly half of the patients tested, diminished excretion following stimulation was noted in one-third of the patients. No correlation was found between the response to HCG and the phenotypes of the patients investigated and mean post-stimulation daily excretion of 11β-hydroxyaetiocholanolone was not significantly different from the basal excretion.

Ratio of 11β-hydroxyandrosterone to 11β-hydroxyaetiocholanolone

Although the stereoisomers 11β-hydroxyandrosterone and 11β-hydroxyaetiocholanolone are structurally similar, they arise from diverse origins. 11β-Hydroxyandrosterone is formed mainly from the metabolism of adrenal androgens (eg Δ^4-androstenedione) whilst 11β-hydroxyaetiocholanolone arises largely from the metabolism of cortisol (Bradlow et al., 1967). The 11-oxy-17-oxosteroids thus include metabolites of both Δ^4-androstenedione and cortisol.

Normal values for the ratio of urinary 11β-hydroxyandrosterone to urinary 11β-hydroxyaetiocholanolone (11β-OH-A/11β-OH-Ae ratio) have recently been reported by Forsyth (1974) who has shown a gradual rise in the 11β-OH-A/11β-OH-Ae ratio from childhood (when the contribution from cortisol metabolites is greater than adrenal androgen metabolites) and she concludes that this gradual rise reflects the mixed origin of the 11-oxy-17-oxosteroids.

The ratios of the daily urinary 11β-hydroxyandrosterone to the daily urinary 11β-hydroxyaetiocholanolone excretion calculated from the basal excretion and from the excretion on the third day of HCG stimulation are shown in columns G and H respectively of Table 3.XVIII. These ratios are compared to the normal 11β-OH-A/11β-OH-Ae ratios found by Forsyth (1974).

It will be noted from Table 3.XVIII that the basal 11β-OH-A/11β-OH-Ae ratios found in many of the patients investigated are greatly elevated. On stimulation of testosterone synthesis by administration of HCG, however, many of these elevated ratios were reduced to normal.

In order to abolish the age-effect of rising 11β-OH-A/11β-OH-Ae ratios, and to facilitate comparison of individual ratios, the results shown in Table 3.XVIII were divided by the normal value for the 11β-OH-A/11β-OH-Ae ratio. Values thus obtained above unity indicate, therefore, an increased 11β-OH-A/11β-OH-Ae ratio, whilst values less than unity indicate an 11β-OH-A/11β-OH-Ae ratio below normal. The comparison of this index of 11β-OH-A/11β-OH-Ae ratio with phenotype is shown in Table 3.XX

There it will be noted that the small number of determinations of the 11β-OH-A/11β-OH-Ae ratio in the patients investigated, and the diversity of those obtained, prevented further statistical analysis.

In patients of short stature the mean basal 11β-OH-A/11β-OH-Ae ratio is very similar to those found in normal cases by Forsyth (1974). This ratio does not alter following stimulation of testosterone synthesis by

TABLE 3.XX CORRELATION OF THE RATIO OF URINARY 11β-HYDROXYANDROSTERONE TO 11β-HYDROXYAETIOCHOLANOLONE EXCRETION IN THE VARIOUS PATIENTS INVESTIGATED

Phenotype and Number	Index of 11β-OH-A/11β-OH-Ae ratio* (Mean ± 1 standard error)	
	BASAL	DAY 3
Short stature (n = 9)	1.32 ± 0.45	1.24 ± 0.24
Bilateral undescended testes (n = 5)	3.73 ± 1.24	2.69 ± 0.86
Cushing's syndrome (n = 1)	0.98	1.05
Hypogonadism with obesity (n = 1)	24.65	-
Laurence-Moon-Biedl syndrome (n = 1)	1.59	3.83
Precocious puberty (n = 1)	5.79	-

* Ratio of 11β-hydroxyandrosterone/11β-hydroxyaetiocholanolone in patient divided by the normal value of this ratio for age (Forsyth, 1974)

administered HCG. Analysis of individual ratios gives a variable picture. Most patients are found to have a low basal ratio, this being caused by a subnormal daily urinary excretion of 11β-hydroxyandrosterone.

In patients with bilateral undescended testes the mean basal 11β-OH-A/11β-OH-Ae ratio is greatly elevated and falls following stimulation of testosterone synthesis by the administration of HCG. The elevated 11β-OH-A/11β-OH-Ae ratios in these patients (3.7 times normal) are attributed to increased daily urinary 11β-hydroxyandrosterone excretion. As urinary 11β-hydroxyandrosterone excretion reflects adrenal androgen production, it is postulated that in these patients with subnormal 11-deoxy-17-oxosteroid excretion, the adrenal is attempting to compensate for a testicular insufficiency.

The basal 11β-OH-A/11β-OH-Ae ratio in one patient with the Laurence-Moon-Biedl syndrome, in whom bilateral undescended testes and hypogonadism are two of the presenting features, is within the normal range. On stimulation of testosterone production by the administration of HCG however, this ratio is greatly raised, suggesting increased adrenal androgen secretion. As the basal daily urinary excretion of 11β-hydroxyandrosterone and 11β-hydroxyaetiocholanolone are both within the normal range, and the 11-deoxy-17-oxosteroid excretion is reduced, this is yet again indicative of testicular insufficiency and related adrenal compensation.

In a patient with hypogonadism and obesity (protocol 56) the 11β-OH-A/11β-OH-Ae ratio is greatly raised. On analysis, this elevated ratio (approximately 25 times normal) is found to be caused by an extremely diminished daily urinary excretion of 11β-hydroxyaetiocholanolone with a normal daily urinary 11β-hydroxyandrosterone excretion. As 11β-hydroxyaetiocholanolone excretion reflects cortisol secretion, the cortisol production in this patient is probably extremely low. It is

interesting that Van Herle et al. (1967) found very low plasma cortisol levels in 2 out of 43 obese boys they investigated.

The basal 11β-OH-A/11β-OH-Ae ratio in a patient with Cushing's syndrome (in whom obesity and hypogonadism are two of the presenting features) is normal and does not alter following stimulation of testosterone synthesis by administration of HCG. On analysis the normal ratio in this patient (protocol 65) is caused by a greatly raised excretion of 11β-hydroxyandrosterone and greatly raised excretion of 11β-hydroxyaetiocholanolone. These elevated levels indicate adrenal 'hyperfunction'. It is interesting that 11β-hydroxyandrosterone is elevated in this patient, indicating increased synthesis of adrenal androgens as previously found by Jailer et al. (1959) and James (1961) in patients with Cushing's syndrome.

In a patient with precocious puberty (protocol 78) the 11β-OH-A/11β-OH-Ae ratio was greatly raised. This elevated ratio, nearly six times normal, is found on analysis to be caused by raised urinary excretion of 11β-hydroxyandrosterone. This surely indicates increased secretion of adrenal androgens characteristic of this phenotype (Gupta and Zimprich, 1966).

Δ^5-Androstene-3β,17β-diol and the androstanediols

The results for the daily urinary excretion of 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol, Δ^5-androstene-3β,17β-diol and 5α-androstane-3β,17β-diol are shown in Table 3.XXI. There it will be noted that the urinary excretion values of these dihydroxysteroids are much lower than the corresponding urinary 17-oxosteroids. In several patients the daily basal excretion of these steroids is below the sensitivity of the method (approximately 0.5 µg per 24 hours) and their absence can be confidently assumed.

On stimulation with HCG, the daily urinary excretion of Δ^5-androstene-3β,17β-diol and the androstanediols is approximately doubled following HCG stimulation although considerable individual variation is noted.

TABLE 3.XXI THE DAILY URINARY EXCRETION OF 5α-ANDROSTANE-3α,17β-DIOL (5α-DIOL); 5β-ANDROSTANE-3α,17β-DIOL (5β-DIOL); Δ^5-ANDROSTENE-3β,17β-DIOL (Δ^5-DIOL) AND 5α-ANDROSTANE-3β,17β-DIOL (3β-DIOL) BEFORE AND AFTER HUMAN CHORIONIC GONADOTROPHIN

Protocol	Age (Years)	5α-diol Excretion (μg/24 hrs)		5β-diol Excretion (μg /24 hrs)		Δ^5-diol Excretion (μg/24 hrs)		3β-diol Excretion (μg/24 hrs)	
		Basal	Day 3	Basal	Day 3	Basal	Day 3	Basal	Day 3
A	B	C	D	E	F	G	H	I	J
1	0.4	12	26	16	59	ND*	ND	ND	ND
2	2.8	11	56	44	402	ND	ND	113	562
3	3.3	1	80	109	254	29	ND	153	190
4	10.1	9	17	12	20	ND	ND	30	53
5	6.0	1	1	2	1	1	1	1	1
6	12.3	38	8	50	46	8	7	9	9
7	14.8	29	23	140	116	ND	ND	ND	ND
8	8.7	ND	ND	37	13	ND	22	44	4
9	14.0	1	12	51	81	ND	ND	60	1,005
10	8.1	1	3	131	127	4	6	65	31
11	8.5	8	15	90	72	148	22	128	102
13	11.0	1	2	91	50	2	8	12	5
14	11.5	2	3	5	4	7	5	46	39
15	12.2	4	5	49	77	12	16	3	9
16	14.3	1	6	39	40	25	15	18	8
17	15.0	1	3	18	26	1	1	1	2
18	5.4	1	1	61	2	20	10	9	122
19	6.0	2	1	71	19	5	1	7	6

20	8.3	50	286	128	260	58	266	325	360
21	10.1	36	6	153	274	4	19	213	36
22	10.3	4	4	38	21	2	2	23	12
23	11.8	ND	ND	ND	ND	16	6	ND	ND
24	15.0	4	6	48	60	12	25	17	6
25	15.3	32	ND	75	ND	ND	ND	ND	ND
26	4.1	1	1	18	28	ND	ND	1	1
27	6.0	6	6	104	4	18	5	19	1
28	7.3	2	1	68	117	89	54	ND	ND
29	8.2	1	1	5	1	1	1	1	1
30	10.8	4	27	24	55	7	5	16	29
31	10.8	28	14	23	2	3	31	19	5
32	11.0	1	1	11	9	1	1	1	1
33	11.2	8	4	14	15	9	2	7	4
34	11.9	3	5	12	80	13	14	4	33
35	13.5	1	1	35	38	1	28	8	5
36	14.8	1	1	2	2	1	12	1	1
37	15.0	1	3	41	32	1	7	2	1
38	16.1	8	26	26	14	4	1	3	1
39	19.0	15	54	44	45	5	4	10	10
40	4.1	9	5	90	149	22	3	76	96
41	4.2	1	73	57	1,424	2	38	1	2
42	13.0	10	14	132	325	87	36	5	13
43	14.0	7	20	94	125	11	11	4	5

TABLE 3.XXI (continued)

Protocol	Age (Years)	5α-diol Excretion (μg/24 hrs)		5β-diol Excretion (μg/24 hrs)		Δ^5-diol Excretion (μg/24 hrs)		3β-diol Excretion (μg/24 hrs)	
		Basal	Day 3	Basal	Day 3	Basal	Day 3	Basal	Day 3
A	B	C	D	E	F	G	H	I	J
44	14.0	14	40	77	71	1	18	2	4
45	16.0	10	16	69	53	2	4	3	5
46	16.0	21	54	103	93	ND	ND	9	25
47	16.0	16	14	85	292	ND	ND	15	9
48	16.0	16	23	76	96	ND	ND	4	2
49	16.1	25	67	60	101	6	10	89	66
50	17.1	3	10	80	67	1	2	1	1
51	3.8	13	5	78	45	4	5	5	2
52	6.8	4	5	51	163	3	8	3	1
53	10.9	29	30	153	126	ND	ND	83	386
54	11.0	14	ND	308	240	ND	ND	33	9
55	12.6	18	ND	643	5,731	145	51	62	21
56	13.7	6	118	86	264	ND	ND	3	17
57	13.9	6	3	65	29	23	10	ND	ND
58	4.5	4	4	ND	ND	24	48	ND	ND
59	5.4	3	3	28	30	22	23	20	34
60	9.5	5	4	54	12	4	128	16	181
61	9.6	5	12	13	5	ND	ND	6	7
62	10.0	7	ND	21	ND	ND	ND	9	ND

63	10.9	10	18	4	15	4	7	2	2
64	14.4	5	4	37	17	ND	ND	12	21
65	15.0	239	30	243	155	19	10	1	1
66	15.6	230	49	75	58	23	76	53	38
67	20.0	73	61	168	116	216	114	95	59
68	26.0	119	129	114	225	3	3	19	15
69	12.1	223	32	144	192	ND	ND	ND	ND
70	19.9	263	638	440	814	300	197	120	126
71	6.3	50	73	39	37	ND	ND	3	1
72	8.0	189	36	23	10	30	3	42	39
73	15.5	149	ND	9	ND	1	1	33	21
74	8.3	2	4	154	144	11	3	277	67
75	7.0	34	22	116	50	1	1	2	1
76	9.0	21	11	66	106	4	4	65	208
77	9.5	ND	13	ND	56	ND	ND	ND	179
78	5.0	2	172	805	193	1	3	2	8
79	17.2	14	9	70	89	1	2	1	2
80	14.0	1	1	18	20	14	58	1	1

* ND = Not Detectable

Δ^5-Androstene-3β,17β-diol

Very few workers have reported values for the urinary excretion of Δ^5-androstene-3β,17β-diol (Δ^5-diol). Wilson et al. (1961) found a urinary Δ^5-diol excretion of 500 µg per 24 hours in a normal adult male. This is in agreement with Bongiovanni (1966) who recorded a value of 37.7 mg per 100 litres in pooled adult male urine. Shackleton et al. (1968) reported very low levels of Δ^5-diol excretion in neonates (range 2-88 µg per 24 hours) rising to a mean level of 370 µg per 24 hours in adults.

The basal urinary excretion of Δ^5-diol found in this investigation is shown in column G, Table 3.XXI. There it will be noted that the basal urinary Δ^5-diol excretion is extremely low in the majority of the patients investigated. Urinary Δ^5-diol was undetected in 20 patients (25 per cent) and extremely low (<5 µg per 24 hours) in a further 29 cases (37 per cent). Absence of published normal values and a shortage of personal patients reckoned to have a normal urinary Δ^5-diol excretion prevented statistical analysis of these low results.

These very low levels of urinary Δ^5-diol excretion were analysed against the phenotypes of the patients investigated. It is interesting that of the 49 patients with undetected or extremely low levels of urinary Δ^5-diol, 17 (35 per cent) have undescended testes, 19 (39 per cent) are of short stature, whilst many of the remaining patients are indeed hypogonadal (micropenis, microscrotum).

It has previously been shown that patients with bilateral undescended testes have undetectable or significantly subnormal excretion of urinary DHA (the precursor of Δ^5-diol with a similar Δ^5-3β-hydroxysteroid structure). It is therefore probable that such patients with extremely reduced levels of Δ^5-3β-hydroxysteroids have a deficiency of 17,20-desmolase for 17α-hydroxy-pregnenolone (an immediate precursor of DHA). Alternatively, there may be a deficiency of 17β-reductase for DHA, although this is unlikely in these patients since DHA is likewise extremely low.

Patients of short stature have an extremely low excretion of urinary Δ^5-diol and low or low-normal urinary excretion of DHA. As these patients have reduced levels of urinary compounds with the Δ^5-3β-hydroxysteroid structure, it is possible that this results from either a reduced activity of 17,20-desmolase or, more likely, a general endocrinological hypofunctioning secondary to the stature of these patients.

Patients with hypogonadism have previously been shown to have normal excretion of urinary DHA (the 17-oxo precursor of Δ^5-diol). As these patients have extremely reduced excretion of urinary Δ^5-diol, it is postulated that the hypogonadism in these patients is due to a deficiency of 17β-reductase. A deficiency of this enzyme could cause extremely low levels of testosterone synthesis and thus subnormal 'androgenicity'.

Δ^5-Diol, originally thought to be merely a peripheral metabolite of DHA, has since been shown to be an intermediate metabolite in the formation of testosterone from DHA (Baulieu et al., 1963). The source of Δ^5-diol has, for several years, been believed to be the adrenal and Δ^5-diol has been tentatively identified in adrenal vein plasma (Wieland et al., 1965). More recently, however, Δ^5-diol has been identified in high concentration in spermatic venous plasma (Laatikainen et al., 1971) and may thus arise by testicular elaboration (synthetic or metabolic).

It is interesting that the basal urinary Δ^5-diol excretion of the patients investigated correlates with the basal urinary excretion of DHA ($r = 0.233$; $p = 0.042$) but does not correlate with the basal urinary excretion of testosterone ($r = 0.071$; $p = 0.302$). As testosterone arises in the male almost entirely by testicular secretion, and DHA is mainly of adrenal origin, this correlation is consistent with the source of Δ^5-diol being adrenal and not testicular. It is interesting that correlations were also found between urinary Δ^5-diol and the other 3β-metabolites, epiandrosterone ($r = 0.223$; $p = 0.045$) and 5α-androstane-3β,17β-diol

($r = 0.599$; $p < 0.001$). This may be taken as further evidence of a direct 3β-pathway between DHA and epiandrosterone (page 169).

To facilitate further study of the effect of HCG on the urinary excretion of Δ^5-diol the patients investigated were again grouped according to height and pubertal status, and the mean stimulation index calculated for each group. As it was considered unethical to give normal children HCG, patients investigated with 'near-normal' phenotype were again used for comparison.

The urinary excretion of Δ^5-diol by 'near-normal' patients in this investigation is approximately doubled following HCG stimulation, although considerable variation is noted (1.90 ± 0.76). This variation is noted throughout the grouped phenotypes and prevented further statistical analysis, all groups being similar to 'near-normals' ($0.2 < p < 0.5$).

5α-Androstane-3α,17β-diol

A considerable volume of experimental evidence has been accumulated in recent years to suggest that testosterone must be metabolised to several closely-related steroids in androgen-dependent tissues in order to effect a maximal biological stimulus in its target cells. The most important of these metabolites outside the liver is 17β-hydroxy-androstane-3-one (dihydrotestosterone). It has been reported that dihydrotestosterone in the blood does not arise from direct secretion and may therefore reflect events occurring in peripheral target tissues (Ito and Horton, 1971). As 5α-androstane-3α,17β-diol (5α-diol) is the main metabolite of dihydrotestosterone at least in normal individuals (Mauvais-Jarvis et al., 1968) the determination of urinary 5α-diol excretion is of clinical interest.

Since urinary 5α-diol originates not only from testosterone metabolism in target tissues but also from the hepatic catabolism of testosterone and dihydrotestosterone (Mauvais-Jarvis et al., 1970a), the excretion of urinary 5α-diol is compared with that of urinary testosterone glucuronoside

which is formed only in the splanchnic compartment (Horton and Tait, 1966). Recently, Mauvais-Jarvis et al. (1973) have shown that the difference between urinary 5α-diol and testosterone excretion reflects the fraction of testosterone which has been metabolised in extra-hepatic tissues through dihydrotestosterone to 5α-diol. This difference in metabolic activity is therefore a reflection of 'androgenicity'.

The basal urinary excretion of 5α-diol is shown in column C, Table 3.XXI. It will be noted there that the basal urinary excretion of 5α-diol is extremely low in the majority of the patients investigated. Although urinary 5α-diol was not detected in only one patient, extremely low levels ($\leq$ 5 μg per 24 hours) were found in a further 30 cases (39 per cent). Absence of published normal values in children and adolescents, and shortage of patients with normal urinary 5α-diol excretion in this investigation, again prevented statistical analyses of these low results. It should be noted, however, that all patients with extremely low excretion of urinary 5α-diol have clinical symptoms of inadequate androgen status (non-masculinized body form often in association with hypogonadism and delayed puberty).

The basal urinary excretion of 5α-diol was plotted against the basal urinary excretion of testosterone for each patient. This graph is shown in Figure 3.12. It will be noted from this graph that the shaded area represents a urinary excretion of 5α-diol less than that of testosterone. All values above this shaded area therefore represent urinary 5α-diol excretions above those of testosterone, whilst the amount of extra-hepatic formation of 5α-diol from the metabolism of testosterone in androgen-dependent tissues is shown by reference to the scales at the left and right-hand side of the graph.

Mauvais-Jarvis et al. (1973) investigated adult females with idiopathic hirsutism and acne vulgaris, as well as normal males and females,

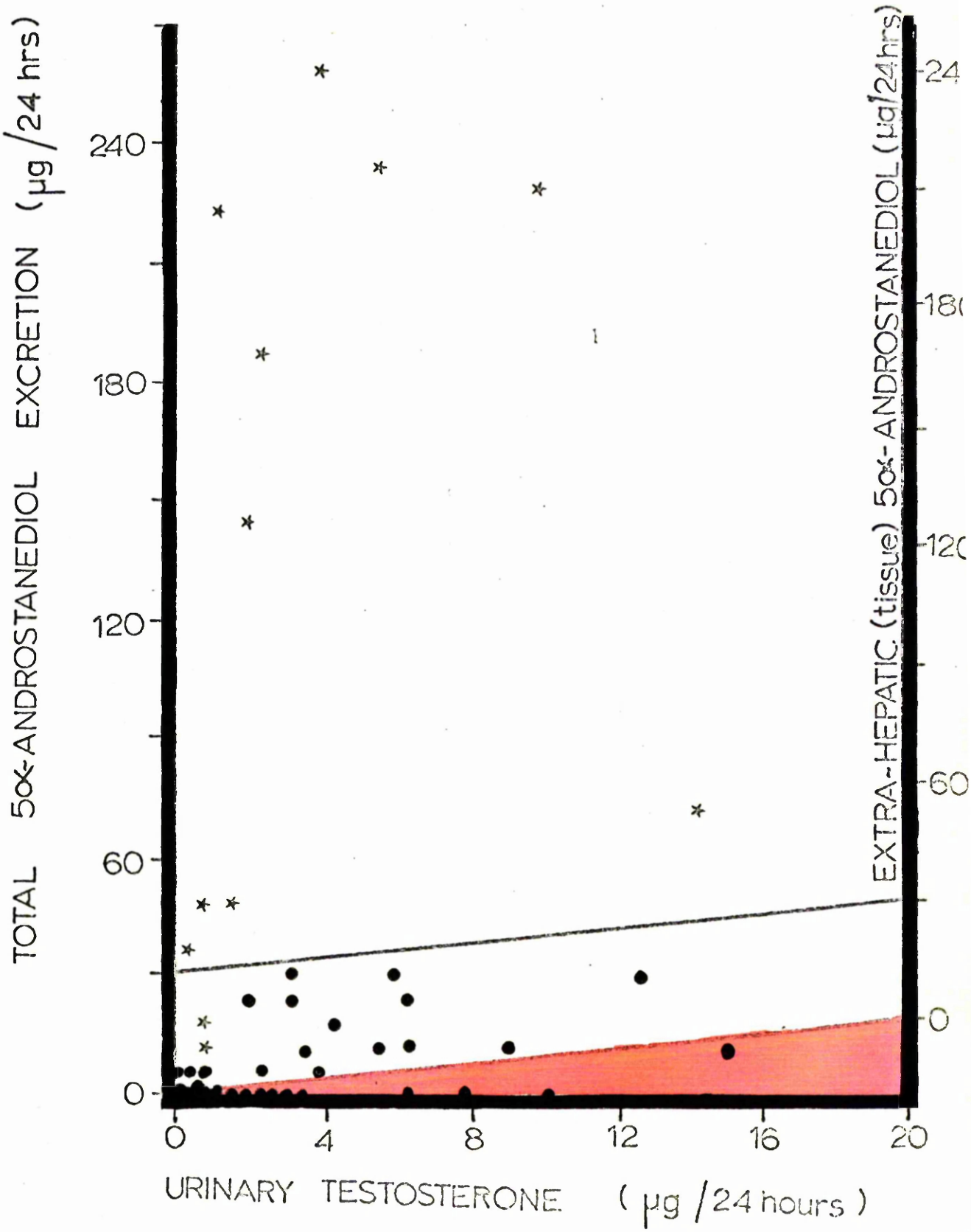


Figure 3.12 The basal urinary excretion of 5α-androstanediol compared to the basal urinary testosterone excretion. The shaded area represents a basal urinary 5α-androstanediol excretion below that of testosterone (probably arising completely from hepatic metabolism of testosterone). See text for explanation.

KEY: ✱ Patients with normal androgen status
● Patients with inadequate androgenicity

with reference to their 5α-diol excretion. Their findings indicate that a daily excretion of extra-hepatic formed 5α-diol not less than 30 µg per day is a constant feature of the androgenic phenomena of hirsutism and acne vulgaris. From my findings relative to the clinical types investigated, I confirm that this lower limit of 30 µg per 24 hours excretion of 5α-diol is always associated with reasonable features of 'paediatric' masculinity. This 5α-diol is of extra-hepatic origin, ie it derives from the metabolism of testosterone in the androgen-dependent tissues.

It will be noted from the graph that the majority of the patients investigated have low levels of urinary 5α-diol excretion arising from extra-hepatic metabolism of testosterone. These low levels are consistent with the lack of androgenicity noted in these patients. These patients are further subdivided when urinary testosterone excretion is considered.

It will be noted from Figure 3.12 that many of the patients with very low levels of urinary 5α-diol excretion have extremely low levels of urinary testosterone excretion. The lack of androgenicity in these patients is therefore probably due to inadequate testosterone production and not to tissue insensitivity. It is interesting that nearly all of these patients have undescended testes and clinical symptoms of 'severe' hypogonadism (rudimentary scrotum and micropenis).

The remaining patients with low levels of urinary 5α-diol excretion have normal levels of urinary testosterone excretion. The lack of androgenicity in these patients is therefore probably due to tissue insensitivity or to a defect in the metabolism of testosterone within the target cells. It is interesting that the majority of these patients are of small stature but without clinical signs of hypogonadism or delayed puberty.

In four patients with the testicular feminization syndrome the urinary excretion of 5α-diol is similar to that of testosterone.

As urinary testosterone is derived mainly from the splanchnic compartment (Horton and Tait, 1966) it would appear that there is no extra-hepatic formation of 5α-diol in these patients. This is in agreement with the results of Mauvais-Jarvis et al. (1968, 1970a, 1973) who found the excretion of 5α-diol was equal to that of testosterone in adult patients with this syndrome.

Likewise, in two patients with Klinefelter's syndrome the urinary excretion of 5α-diol was found to be similar to the urinary excretion of testosterone. It is therefore probable that in these patients there is again no extra-hepatic formation of 5α-diol, and testosterone is not metabolised in the target tissues. In the patient with 47,XXY karyotype this is probably due to inadequate testosterone synthesis, whilst in the 46,XX-male this may be due to tissue insensitivity or a defect in the metabolism of testosterone.

The remaining patients all had levels of urinary 5α-diol excretion above 30 μg per 24 hours and adequate androgen status. It is interesting that two of these patients are females, and have XO/XY chromosomal mosaicism with clinical signs of increased androgenicity, ie enlarged clitoris, laryngeal development.

The basal urinary excretion of 5α-diol is above the normal adult male range (133 $\pm$ 25 μg per 24 hours; Mauvais-Jarvis et al., 1973) in five patients. Three of these patients were in early puberty at the time of investigation and it is therefore probable that 5α-diol production increases dramatically during puberty. During early puberty testosterone synthesis and excretion are still low, yet the growth of the testis is maximal. It is therefore postulated that 5α-reduced metabolites of testosterone (or other androgens) may play an important role in initiation or maintenance of this high rate of testicular growth. During adolescence, the rate of testosterone synthesis increases and the formation of 5α-reduced metabolites is therefore suppressed (Oshima et al., 1970).

It is interesting that in this investigation the urinary excretion of 5α-diol was significantly correlated with age ($r = 0.307$; $p = 0.003$), bone age ($r = 0.339$; $p = 0.002$), pubertal status ($r = 0.310$; $p = 0.005$), gonadal status ($r = 0.345$; $p = 0.002$) and the urinary excretion of testosterone ($r = 0.234$; $p = 0.026$). Further significant correlations were also found between the urinary excretion of 5α-diol and the individual and grouped 17-oxosteroids and individual androstanediols.

To facilitate further study of the effect of administered HCG on the urinary excretion of 5α-diol the patients investigated were again grouped according to height and pubertal status, and the mean stimulation index calculated for each group. These indices are shown in Table 3.XXII.

It will be noted from this table that the urinary excretion of 5α-diol by 'near-normal' patients in this investigation is approximately trebled after HCG stimulation, although again considerable individual variation is noted. Those patients with 'near-normal' phenotype are again used for comparison of the various phenotypes investigated.

Patients with short stature have a normal increase in 5α-diol excretion following HCG. This is further evidence for normal testicular function in these patients, low levels of excretion probably being related to the small body size.

Patients with delayed puberty have a low or normal basal urinary excretion of 5α-diol and have a small increased 5α-diol excretion following HCG stimulation. This response, only half that of 'near-normal' patients, is just significantly subnormal ($0.02 < p \leq 0.05$). Likewise, patients with severe hypogonadism, having similar basal 5α-diol excretion, have a very poor increase following HCG. This response (1.12 ± 0.16), only one-third that of 'near-normal' patients, is very significant ($0.002 < p < 0.005$). These results are, of course, in keeping with the poor androgenicity of such patients (Mauvais-Jarvis *et al.*, 1973).

TABLE 3.XXII EFFECT OF ADMINISTERED HUMAN CHORIONIC GONADOTROPHIN ON THE DAILY URINARY EXCRETION OF 5α-ANDROSTANE-3α,17β-DIOL BY DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)
'Near-normal' phenotype (n = 5)	3.19 ± 0.58
Short stature (n = 12)	3.08 ± 0.98
Delayed puberty (n = 4)	1.52 ± 0.42**
Unilateral undescended testes (n = 4)	1.35 ± 0.24**
Hypogonadism (n = 18)	1.12 ± 0.16**
Bilateral undescended testes (n = 16)	0.97 ± 0.11**

* Stimulation Index = $\frac{\text{Urinary } 5\alpha\text{-diol (3rd day)}}{\text{Basal urinary } 5\alpha\text{-diol}}$

** Significantly below 'near-normal'

Four patients with unilateral undescended testes have extremely low basal urinary excretion of 5α-diol and have a low increase following HCG. This response is significantly below that seen in 'near-normal' patients ($0.02 < p < 0.05$). However, patients with bilateral undescended testes, also having a very low basal excretion of 5α-diol, have no response to HCG, the post-stimulation excretion being generally equal to the basal excretion of 5α-diol. This response is significantly different ($0.001 < p < 0.002$) from that of 'near-normal' patients. Such low levels of urinary 5α-diol excretion with poor response to HCG suggest insignificant 'extra-hepatic' metabolism of testosterone and are in keeping with the poor clinical features.

5β-Androstane-3α,17β-diol

Robel et al. (1966a) have shown that most of the urinary excretion of 5β-androstane-3α,17β-diol (5β-diol) arises from the metabolism of testosterone glucuronoside whilst only a minor fraction is formed from the metabolism of free testosterone. As testosterone glucuronoside circulates in plasma in concentrations similar to that of testosterone (Burger et al., 1964) it is possible that this metabolite is an important factor in the transport of testosterone.

Although 5β-metabolites of androgens are generally believed to be inactive (Baulieu, 1970) it is perhaps presumptuous to assume that only 5α-reduction of testosterone is relevant to its action in all tissues. Levere and Granick (1967) have shown that red blood cell formation is selectively stimulated by 5β-androstane metabolites, whilst recently Parsons (1970) has shown that 5β-diol is the major metabolite of testosterone in chick blastoderm at a time commensurate with the initiation of erythropoiesis.

As 5β-diol is the main metabolite of testosterone glucuronoside and the urinary excretion of this metabolite exceeds that of 5α-diol

(Mauvais-Jarvis and Baulieu, 1965), the determination of urinary excretion of 5β-diol may be of clinical interest.

The basal urinary excretion of 5β-diol is shown in column E, Table 3.XXI. It will be noted there that the basal urinary excretion of 5β-diol exceeds that of 5α-diol. Urinary 5β-diol excretion was undetected in only one patient, whilst extremely low levels (< 5 μg per 24 hours) were only found in a further three patients. Although Berthou et al. (1971) have recently reported normal values for the urinary excretion of 5β-diol in adults, the absence of published normal values in children and adolescents and the low number of patients with normal urinary excretion of 5β-diol in this investigation again prevent statistical analyses of these low results. It should be noted, however, that these patients with extremely low excretion of urinary 5β-diol have clinical symptoms of inadequate genital development (microgenitalia and hypospadias).

Having investigated a large number of male patients with gonadal problems, it is possible to associate the urinary excretion of 5β-diol with the development of the external genitalia, good genital development accompanying high urinary excretion of 5β-diol. By reference to the clinical observations in the patients investigated and the results of Berthou et al. (1971) on normal males and females, a minimum daily urinary excretion of 50 μg per 24 hours has been taken as the lower limit of 5β-diol excretion commensurate with normal genital development.

The basal urinary excretion of 5β-diol correlates with the basal urinary excretion of testosterone ($r = 0.405$; $p < 0.001$). This correlation is in agreement with testosterone glucuronoside (the major fraction of urinary testosterone), being the precursor of 5β-diol. The basal urinary excretion of 5β-diol was therefore compared to that of testosterone.

The basal urinary excretion of 5β-diol was plotted against the basal urinary excretion of testosterone for each patient. This graph is shown

in Figure 3.13. It will be noted from this graph that all patients with a low basal urinary excretion of 5β-diol have very low levels of urinary testosterone. All of these patients have inadequate genital development and many show clinical symptoms of severe hypogonadism. The poor genital status observed in these patients is therefore probably due to inadequate testosterone production.

It should be noted that the low basal urinary excretions of 5β-diol and testosterone found in these patients are not related to age, with the exception of two young patients with the testicular feminization syndrome. These two patients (protocols 1 and 2) have normal levels of urinary 5β-diol excretion for age, and will be considered later.

Of the remaining patients with low levels of urinary 5β-diol excretion, 11 (38 per cent) show severe hypogonadism, 9 (31 per cent) have bilateral undescended testes with associated hypogonadism, 8 (28 per cent) are of short stature, whilst the remaining patient (protocol 4) presented with the complete form of the testicular feminization syndrome.

The remaining patients all have basal urinary excretions of 5β-diol above 50 μg per 24 hours and normal genital development with the exception of 7 patients. Two patients with XO/XY mosaicism (protocols 6 and 7), associated with gross clitoral enlargement, have normal male levels of urinary 5β-diol excretion. It is interesting that both of these females showed clitoral enlargement, one having a phallus (before amputation) of 3-4 cm. Similarly, the basal urinary excretion of 5β-diol is within the normal male range in three patients with the testicular feminization syndrome (protocols 1, 2 and 3). It is interesting that clinically two of these patients show mild clitoral enlargement and it is reasonable to assume that these three 'girls' will show further masculinisation at puberty and therefore have the incomplete form of this syndrome.

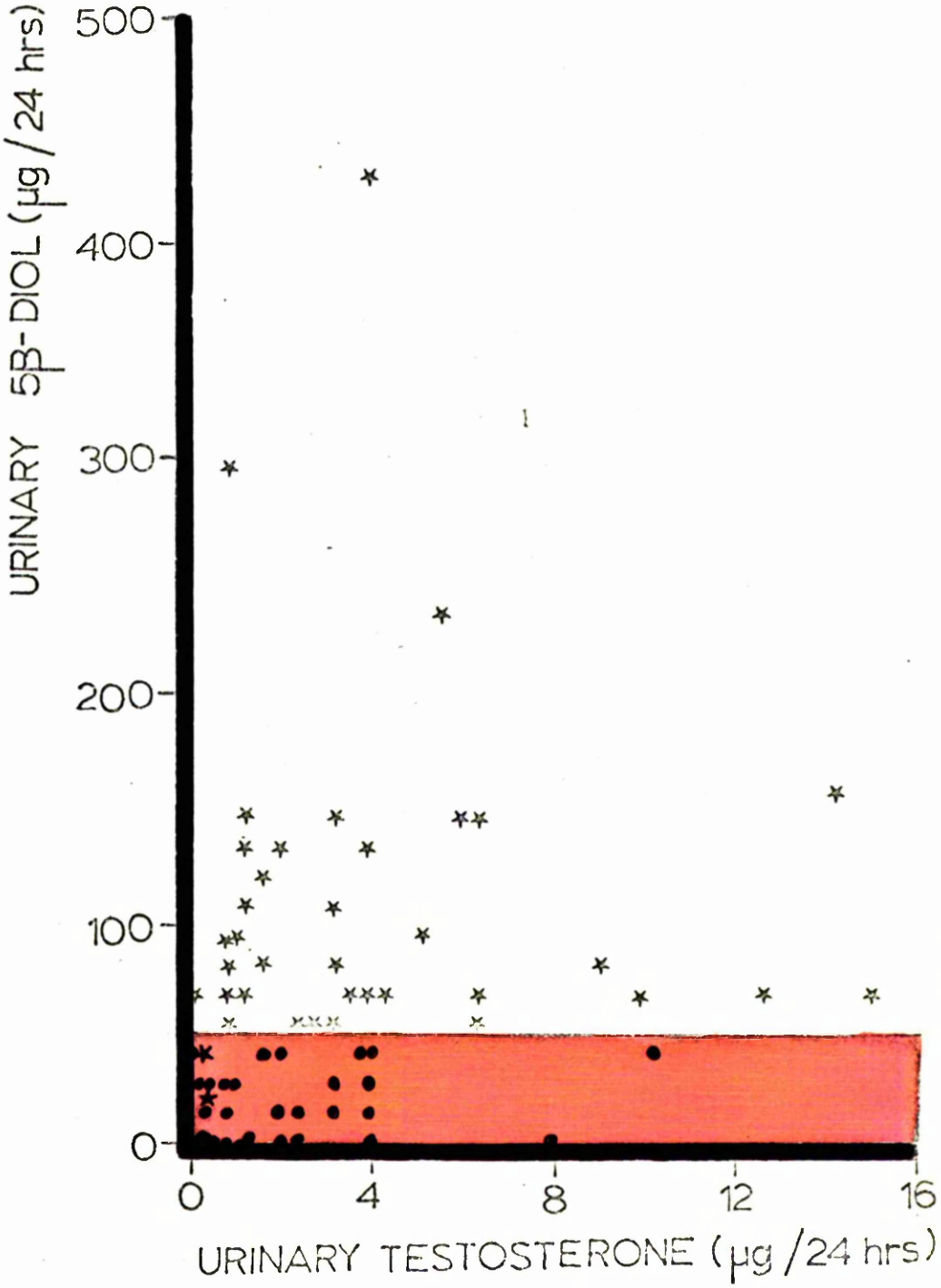


Figure 3.13 The basal urinary excretion of 5β-androstanediol compared to the basal urinary testosterone excretion. Note normal masculine genital development in all patients with a urinary excretion of 5β-androstanediol above 50 µg per 24 hours.

KEY: ✱ Patients with normal (masculine) genital developmen
● Patients with microgenitalia

The remaining two patients have a normal male phenotype, bilateral undescended testes and poor genital development. Their basal urinary excretion of 5β-diol is, however, within the normal range. It is interesting that on application of testosterone cream to the genital area in these patients extremely good results were obtained (ie penile growth and appearance of pubic hair). 5β-Diol may therefore have a prognostic value.

As expected from the above results, the basal urinary excretion of 5β-diol was significantly correlated with bone age ($r = 0.199$; $p = 0.046$), genital development ($r = 0.332$; $p = 0.003$) and the urinary excretion of testosterone ($r = 0.405$; $p < 0.001$). Further correlations were noted between the urinary excretion of 5β-diol and the individual androstanediols, androsterone and epiandrosterone.

On facilitate study of the effect of HCG on the urinary excretion of 5β-diol the patients were again grouped according to phenotype and the mean stimulation index calculated for each group. These indices are shown in Table 3.XXIII.

It will be noted from this table that the urinary excretion of 5β-diol by 'near-normal' patients in this investigation is increased approximately 2.5 times after HCG stimulation, although again considerable individual variation is noted. These patients with 'near-normal' phenotype are again used for comparison of the various phenotypes investigated.

Patients with unilateral undescended testes, having a basal urinary 5β-diol excretion ranging from very high to very low, have a normal, although extremely variable, response following HCG administration.

Patients with delayed puberty also have a variable basal urinary 5β-diol excretion which is increased poorly following HCG. This response (1.15 ± 0.27) is statistically significantly below that of 'near-normal' patients ($0.002 < p < 0.005$) and is in agreement with the retarded genital growth of such patients.

TABLE 3.XXIII EFFECT OF ADMINISTERED HUMAN CHORIONIC GONADOTROPHIN ON THE DAILY URINARY EXCRETION OF 5β-ANDROSTANE-3α,17β-DIOL BY DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)
'Near-normal' phenotype (n = 7)	2.57 ± 0.23
Unilateral undescended testes (n = 6)	2.41 ± 1.33
Delayed puberty (n = 5)	1.15 ± 0.27**
Short stature (n = 26)	1.14 ± 0.16**
Bilateral undescended testes (n = 14)	1.00 ± 0.26**
Hypogonadism (n = 21)	0.86 ± 0.15**

* Stimulation Index = $\frac{\text{Urinary 5β-diol (3rd day HCG)}}{\text{Basal urinary 5β-diol}}$

** Significantly below 'near-normal' patients

Patients of short stature, having a low basal urinary 5β-diol excretion, have a lowered response following HCG. This response, less than half of that of 'near-normal' patients, is highly significantly subnormal ($p < 0.00005$) and cannot be explained by the short stature alone. However many of these patients with short stature did have small external genitalia.

Patients with bilateral undescended testes, having very low basal urinary excretion of 5β-diol, have no response following HCG, the excretion of 5β-diol being the same as basal excretion. This lack of response is very significant ($0.0002 < p < 0.0005$) and similar to that noted with 5α-diol.

Hypogonadal patients, having low or normal levels of urinary excretion of 5β-diol, have decreased 5β-diol excretion following HCG. This response is highly significantly below that of 'near-normal' patients ($p < 0.00005$). This decreased excretion may reflect changes in the hepatic metabolism of testosterone.

5α-Androstane-3β,17β-diol

5α-Androstane-3β,17β-diol (3β-diol) has been shown to be a metabolite of testosterone in the human prostate benignly hypertrophied (Chamberlain et al., 1966). Baulieu et al. (1968) studied the effects of 3β-diol on tissue culture of rat prostate and reported that this metabolite maintained growth and secretion of the epithelial cells. The physiological importance of 3β-diol has since been emphasized by Baulieu (1970) and Robel et al. (1971).

3β-Diol has been identified as a sulpho-conjugate in plasma (Cronholm and Sjovall, 1968), as both free and as a sulpho-conjugate in human faeces (Janne et al., 1971) and recently in urine following β-glucuronidase hydrolysis (Berthou et al., 1972).

The basal urinary excretion of 3β-diol by the patients in this investigation is shown in column I, Table 3.XXI. It will be noted there that this is very low in the majority. Urinary 3β-diol excretion was undetected in 6 patients (8 per cent), whilst extremely low levels

(< 5 µg per 24 hours) were found in a further 25 cases (33 per cent). Although Berthou et al. (1972) have recently reported normal values for the urinary excretion of 3β-diol in 5 adults, the absence of published normal values for children and adolescents, and the low number of patients with normal urinary excretion of 3β-diol in this investigation, again prevented statistical analyses of these low results. It should be noted, however, that 7 patients (23 per cent) with extremely low urinary excretion of 3β-diol have bilateral undescended testes, whilst 12 patients (39 per cent) have either severe hypogonadism or delayed puberty.

The basal urinary excretion of 3β-diol of the patients investigated correlates with the basal urinary excretion of 5α-diol ($r = 0.354$; $p < 0.001$). No correlation was found between the basal urinary excretion of 3β-diol and the basal urinary excretion of testosterone ($r = 0.009$; $p = 0.470$). This is in agreement with the results of Mauvais-Jarvis et al. (1970b) who have shown that 80 per cent of administered 3β-diol was metabolised in vivo to 5α-diol (the 3α-epimer) and androsterone and may explain the low urinary excretion of 3β-diol.

The basal urinary excretion of 3β-diol was plotted against the basal urinary excretion of 5α-diol for each patient. This graph is shown in Figure 3.14. It will be noted that many of the patients with very low levels of urinary 3β-diol excretion have also extremely low levels of urinary 5α-diol excretion. As 5α-diol and 3β-diol are metabolites of testosterone within the target cells, it is probable that many of these patients lack such a metabolism and have either a tissue insensitivity to testosterone or alternatively primary testicular failure. This is no doubt the case in two of these patients with the testicular feminization syndrome (protocols 1 and 4) and a further six patients with primary testicular failure (protocols 18, 19 and 22-25). However the majority of these patients with low excretion of both 5α-diol and 3β-diol are of

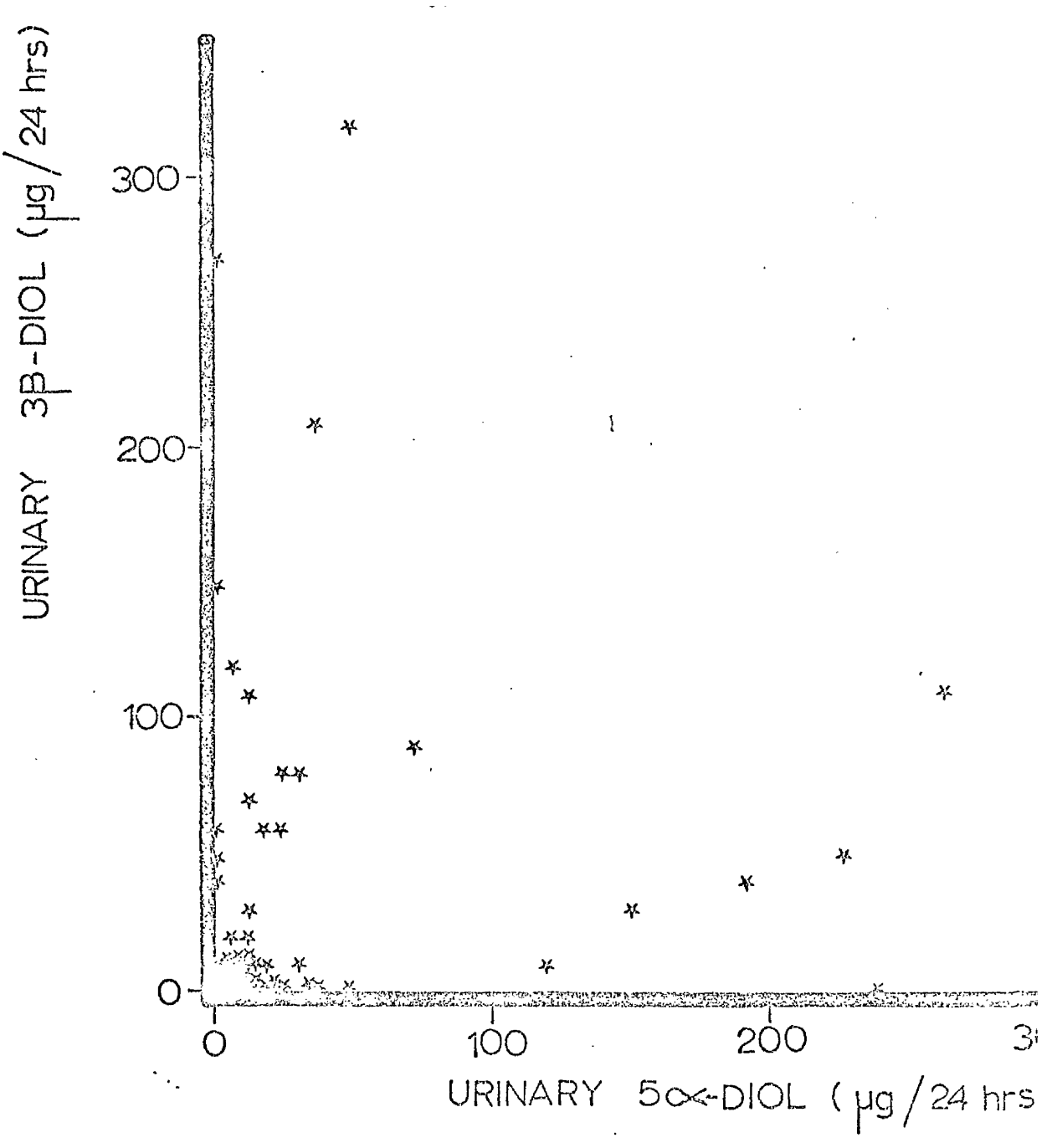


Figure 3.14 The basal urinary excretion of 5α-androstane-3β,17β-diol (3β-diol) compared with the basal urinary 5α-androstanediol (5α-diol) excretion in individual patients. See text for explanation.

short stature and in most cases their low excretion is probably related to their small body size.

The remaining patients with low levels of urinary 5α-diol excretion have normal or elevated urinary excretion of 3β-diol. It is interesting that nearly all of these patients have severe hypogonadism (rudimentary scrotum, micropenis) and many have undescended testes.

The mean ratio of the urinary excretion of 5α-diol to the urinary excretion of 3β-diol (the 3α/3β epimers ratio) in these patients is 0.24 ± 0.20 (SD) which is statistically highly significantly different ($p < 0.001$) from that found by Berthou et al. (1972) in three normal adult males (5.09 ± 0.82).

These patients have been shown to have extremely low urinary excretion of testosterone which may reflect inadequate testicular production of testosterone. It has previously been postulated that when general tissue metabolism cannot utilize testosterone because of inadequate production, then DHA becomes the principal anabolic C19 hormone (Hamilton, 1974). DHA production thus increases in such patients and the urinary excretion of epiandrosterone increases correspondingly. Under these conditions the urinary excretion of 3β-diol (a metabolite of epiandrosterone) will also increase as noted in these patients.

The remaining patients all have normal urinary excretion of 3β-diol as judged by a normal ratio of the urinary excretion of 5α-diol to that of 3β-diol. The mean 3α/3β epimer ratio in these patients is 4.48 which is in good agreement with that found in normal adult males by Berthou et al. (1972) and the conversion of plasma 5α-dihydrotestosterone to 5α-diol/3β-diol ratio of 4.90 in normal adult males found by Mahoudeau et al. (1971).

To facilitate further study of the effect of HCG on the urinary excretion of 3β-diol the patients investigated were again grouped according to height and pubertal status and the mean stimulation index calculated

for each group. Patients investigated with 'near-normal' phenotype were again used for comparison. These indices are shown in Table 3.XXIV.

It will be noted from this table that the urinary excretion of 3β-diol by 'near-normal' patients in this investigation is increased approximately 1.4 times after HCG stimulation.

Patients with short stature, having very low basal urinary 3β-diol excretion, have a good response to HCG administration. This response (1.58 ± 0.18) is very similar to that of 'near-normal' patients and evidence for normal testicular function in these patients.

Patients with delayed puberty, having normal basal urinary excretion of 3β-diol, have a normal response to HCG administration. However, patients with hypogonadism, having normal or raised urinary excretion of 3β-diol, have a decreased excretion of 3β-diol following HCG. This decrease is significantly below that of 'near-normal' patients ($0.01 < p < 0.02$) and this, in combination with the raised urinary excretion of epiandrosterone, is further evidence for a partial deficiency of 17β-reductase previously postulated in such patients (page 187).

Patients with unilateral undescended testes, having very low basal urinary excretion of 3β-diol, have no response to HCG administration. Furthermore patients with bilateral undescended testes have a decreased excretion of 3β-diol following HCG. This decrease is again significantly below that of 'near-normal' patients ($0.01 < p < 0.02$) but not significantly below that of patients with unilateral undescended testes. These results, again in combination with the elevated urinary excretion of epiandrosterone, suggest a partial deficiency of 17β-reductase in many of these patients with undescended testes.

Two patients with severe gynaecomastia, having elevated urinary excretion of 3β-diol, have extremely raised urinary excretion of 3β-diol following HCG. The response to HCG in these patients (10.70 ± 6.05) is consistent with my

TABLE 3.XXIV EFFECT OF ADMINISTERED HUMAN CHORIONIC GONADOTROPHIN ON THE DAILY URINARY EXCRETION OF 5α-ANDROSTANE-3β,17β-DIOL BY DIFFERENT PHENOTYPES

Phenotype and Number	Stimulation Index* (Mean ± 1 standard error)
'Near-normal' phenotype (n = 5)	1.40 ± 0.20
Short stature (n = 26)	1.58 ± 0.18
Delayed puberty (n = 5)	1.24 ± 0.20
Unilateral undescended testes (n = 6)	1.00 ± 0.35**
Hypogonadism (n = 18)	0.78 ± 0.12**
Bilateral undescended testes (n = 10)	0.74 ± 0.14**

* Stimulation Index = $\frac{\text{Urinary 3β-diol (3rd day HCG)}}{\text{Basal urinary 3β-diol}}$

** Significantly below 'near-normal' patients

postulate of a partial deficiency of 3β-hydroxysteroid dehydrogenase in these patients.

Numerous results and statistical calculations have been presented in this chapter. These results will now be discussed and related more particularly to the clinical condition of the patients. Indeed an attempt will be made to explain the clinical findings in relation to both how much testosterone is elaborated and how the body responds at the tissue level to it.

CHAPTER 4

D I S C U S S I O N

It is now generally accepted that the determination of the total urinary 17-oxosteroid excretion is of limited use in assessing testicular function. Only 24 per cent of testosterone secreted by the testes is metabolized to such compounds (Hall et al., 1974) and the testicular contribution to the total 17-oxosteroids is only half that of the adrenal component.

In this investigation quantitation of the total urinary 17-oxosteroids by the Zimmermann reaction gave extremely variable results which in the majority of patients were greater than the sum of individual 17-oxosteroids quantitated by flame ionization detection following gas liquid chromatography (GLC-FID). The difference between these results, the non-steroid chromogenic material, is often considerable and is an indication of the non-specificity of the Zimmermann reaction. This non-specificity has been noted previously by Goldzieher and Axelrod (1962) and more recently quantitated by Matthijssen and Goldzieher (1971).

The determination of the urinary total 17-oxosteroids as a routine clinical test is of little value other than to indicate gross abnormalities in excretion. Nonetheless very low excretion of these compounds was found in older patients of short stature and patients with delayed puberty and this is likely to be significant. However, the quantities measured gave indication of the relative steroid mass for the loading of TLC and GLC systems.

Large variation was found in the volumes of serial 24-hour urine specimens despite the well organised ward conditions for urine collection. This variation does not appear to be related to age but is probably due in part to incomplete collection. The daily urinary creatinine excretion was found to be significantly more constant for individual patients than the 24-hour urine volume although significant variation still persisted. This residual variation may be due in part to diurnal rhythm in the case of incomplete collection.

Several investigators have noted high variability in the daily urinary creatinine excretion and have attempted to explain this (Wray and Scott Russell, 1960; Doolan et al., 1962; Paterson, 1967; Scott and Hurley, 1968; Curtis and Fogel, 1970; Turner and Cohn, 1975). Alterations in dietary protein have been implicated as a source of variability but frequently refuted or held to be of minimal importance (Folin, 1905b; Cramér et al., 1967; Curtis and Fogel, 1970). Physical activity, unless extreme, also seems to have little effect on creatinine variation (Tanner, 1968; Chattaway et al., 1969). Similarly urinary volume has little effect under normal conditions (Cramér et al., 1967; and this investigation) 15 ml per kilogram per day being adequate for excretion. In this investigation no effect of HCG on the daily creatinine excretion was noted either over the short period of test (6,000 I.U. daily for three days) or on longer therapeutic usage (1,500 I.U. alternate days for one month, protocol 79).

Several groups of workers have found the daily urinary creatinine excretion more constant than the 24-hour urine volume (Doolan et al., 1962; Bleiler and Schedl, 1962; Paterson, 1967; Curtis and Fogel, 1970) whilst others have disputed this (Cramér et al., 1967; Scott and Hurley, 1968; Chattaway et al., 1969). This constancy was used throughout this investigation as a measure of the completeness of collection of 24-hour urine samples. Applegarth et al. (1968) have previously stressed the usefulness of creatinine excretion from their study of amino acids in children.

When the daily urinary creatinine excretion was plotted against age an interesting correlation was found. The urinary creatinine excretion of patients with normal stature was found to rise gradually during the prepubertal period and increase rapidly in parallel with the normal growth spurt at puberty.

The urinary creatinine excretion in patients of short stature was significantly below, although parallel to, the normal excretion whilst in patients with delayed puberty, not having a pubertal growth spurt, the urinary creatinine excretion rose gradually and approached the much lower excretion found in the older patients of short stature. Patients with precocious puberty and advanced bone age had an elevated urinary creatinine excretion. It is generally accepted that creatinine is a product of protein catabolism and that the urinary excretion of creatinine is significantly related to lean body mass, active protoplasmic mass, weight and surface area (Doolan et al., 1962; Bleiler and Schedl, 1962; Turner and Cohn, 1975). The results of this investigation are in agreement with the established concepts that the urinary creatinine excretion is significantly related to stature (Graystone, 1968).

Those patients with abnormal urinary creatinine excretion also had an abnormal urinary excretion of both grouped and individual steroids. When these results of steroid excretion were adjusted to a normal creatinine excretion many of these results, particularly those from patients of short stature, were adjusted to the normal range whilst abnormal results still persisted in several patients with precocious puberty, delayed puberty and hypogonadal patients of short stature. Thus many patients with short stature alone had a normal urinary 17-oxosteroid excretion when compared to their creatinine output. Patients with delayed puberty and hypogonadal patients had a subnormal urinary 17-oxosteroid excretion when compared to their creatinine output, whilst two patients with precocious puberty had an elevated urinary steroid excretion even after correction for a normal creatinine output. These results suggest that many of the subnormal results of steroid excretion in patients of short stature alone relate to the smaller endocrine gland size with reduced secretion of anabolic hormones.

Conversely, patients with persisting abnormal urinary excretion of androgens and androgen metabolites after correction to a normal creatinine excretion probably have endocrine disorders of the testes or adrenals. Comparison of the results of urinary steroid analyses with the urinary creatinine excretion may therefore have diagnostic use in the study of patients with growth disorders in association with abnormal endocrine function.

It will be noted that in my large group of patients the urinary excretion of testosterone is significantly lower than normal in patients of short stature, bilateral undescended testes, hypogonadism and delayed puberty. Diminished urinary testosterone excretion has been reported previously by Schmidt and Starcevic (1967) in delayed puberty, Moxham and Nabarro (1968) in hypogonadism, Scurry et al. (1971) in hypogonadism and delayed puberty and more recently in prepubertal boys with cryptorchidism, hypogonadism or delayed puberty by Rudd et al. (1973).

My finding of normal or elevated levels of plasma testosterone in these patients is surprising. Most investigators of like patients have found decreased concentration of plasma testosterone (Rivarola et al., 1970; Anderson et al., 1972; Perheentupa et al., 1972; Dessypris and Adlercreutz, 1972; Boyar et al., 1973; Stewart-Bentley and Horton, 1973; Rudd et al., 1973; Walsh et al., 1976). Over-estimation of plasma testosterone in these patients reported in this thesis is unlikely as the same methods of purification, detection and quantitation were employed for both plasma and urinary testosterone determinations. Moreover, very low concentrations of plasma testosterone have been detected (15 ng per 100 ml) and very good agreement has been found between these results and results obtained when duplicate samples were analysed by a competitive protein binding assay (five patients).

Despite controversy in the past, recent studies have demonstrated a circadian rhythm of plasma testosterone levels (DeLacerda et al., 1973;

Boyar et al., 1975; Southren and Gordon, 1975; Scholler et al., 1975). Highest levels have been found about the time of awakening whilst lowest levels are found in the evening. However, the amplitude of this rhythm is small (10-25 per cent) and DeLarcerda et al. (1973) have found that only 20 per cent of the total variance of hourly integrated testosterone levels was due to a time effect. Moreover mean daily levels of plasma testosterone were attained by 11 am (Scholler et al., 1975), the time of sampling used throughout this investigation.

The circhoral rhythm of plasma testosterone levels has much greater amplitude (Baker et al., 1975). A close temporal relationship between the nocturnal rises of LH and testosterone in pubertal boys has been found by Boyar et al. (1975) suggesting that the Leydig cells respond rapidly to LH. No significant correlation has been found between LH and testosterone levels in data obtained at short time intervals (Alford et al., 1973). However, these workers found such a correlation between testosterone levels and LH levels in the preceding sample when using continuous sampling over three hours. This suggests that the testicular response may be delayed two to four hours which is in agreement with the delay of four to six hours noted in testosterone rise following intravenous HCG administration (Maurer et al., 1973).

Circhoral rhythm of plasma testosterone levels could explain some of the high levels of plasma testosterone noted in this thesis. However, more frequent sampling was considered unethical in the young patients investigated.

Doering et al. (1975) have recently found a further variation in plasma testosterone levels. They described a circatrigintan rhythm of 23 days (range 8-30 days) with an amplitude similar to the circadian rhythm. Such a rhythm, if present in children, should not affect the plasma testosterone levels over the short period of test (4-5 days) but again may explain some of the high plasma testosterone levels.

Normal or elevated plasma testosterone in combination with subnormal urinary testosterone excretion can be explained by an increased binding in plasma to testosterone-binding β-globulin (TeBG). High plasma testosterone levels due to increased TeBG levels have been reported in pregnancy and in hyperthyroidism (Vermeulen et al., 1969; Pohlman et al., 1969; Vermeulen and Verdonck, 1972). Such elevated levels of plasma testosterone are not usually accompanied by virilization as only the free non-protein-bound testosterone fraction is biologically active. Vermeulen and Verdonck (1972) also reported increased levels of TeBG-bound testosterone in prepubertal boys and in adult hypogonadal males. It can readily be envisaged that the plasma testosterone in these hypogonadal prepubertal boys is probably nearly quantitatively bound to TeBG. Vermeulen et al. (1969) observed an inverse correlation between the metabolic clearance rate of testosterone (MCR^t) and the binding capacity and binding index of TeBG. They postulated that testosterone bound to TeBG is not as readily metabolized as the unbound hormone. Further work by Vermeulen and Verdonck (1972) has shown a significant correlation between the MCR^t and the apparent free testosterone concentration. Moreover, these authors also observed that increased binding of testosterone to TeBG was characterized by a relative increase of 5β over 5α metabolites of testosterone and a decrease in its 17β-hydroxy metabolites. They relate this change in metabolism to a decreased target organ metabolism of testosterone.

Subnormal urinary excretion of testosterone in combination with normal or elevated plasma testosterone may arise alternatively by tissue insensitivity to testosterone. Under these conditions there is less degradation of circulating testosterone secondary to a poor utilization of testosterone by testosterone-dependent tissues. If this were so, then free testosterone can only be cleared by urinary loss or liver metabolism.

It is now generally accepted that testosterone must be metabolized to dihydrotestosterone, 5α-androstane-3α,17β-diol (5α-diol) and 5α-androstane-3β,17β-diol (3β-diol) in order to exert a maximal biological stimulus in the classical androgen-dependent organs (Baulieu et al., 1968; Baulieu, 1970; 1973). The most important of these metabolites outside the liver is dihydrotestosterone. Ito and Horton (1971) reported that dihydrotestosterone in the blood does not arise from direct secretion and may therefore reflect events occurring in the peripheral target tissues. Giorgi et al. (1971), using a superfusion technique, elegantly demonstrated the formation and intracellular retention of radioactively labelled 5α-dihydrotestosterone from radioactively labelled testosterone in biopsies of human prostate. Shimazaki et al. (1966) located the prostate 5α-reductase in the microsomes and noted its absolute specificity for NADPH. Bruchovsky and Wilson (1968) found that up to half of the 5α-reductase is associated with the nuclear fraction in accessory sexual glands and they demonstrated the formation of androstanediols in the soluble cytoplasmic fraction of these tissues. Frederiksen and Wilson (1971) studied the kinetic properties of the 5α-reductase in rat prostate in detail and from consideration of the levels of testosterone, NADPH and the total enzymic activity of the gland they concluded that the reaction is limited only by the levels of available testosterone. As 5α-diol is the main metabolite of dihydrotestosterone, at least in normal individuals (Mauvais-Jarvis et al., 1968), the determination of urinary 5α-diol excretion is of clinical interest.

However, since urinary 5α-diol originates not only from testosterone metabolism in target tissues but also from the hepatic catabolism of testosterone and dihydrotestosterone (Mauvais-Jarvis et al., 1970a), the urinary excretion of 5α-diol must be compared to that of urinary testosterone glucuronoside which is formed only in the splanchnic compartment (Horton and Tait, 1966). The difference between the urinary

5α-diol excretion and testosterone therefore reflects target organ metabolism of testosterone and is an index of androgenicity (Mauvais-Jarvis et al., 1973).

We (the writer and Dr William Hamilton; Hamilton 1974a) have confirmed that 5α-diol is a good reflection of androgenicity and have found a lower limit of extra-hepatic formed 5α-diol of 30 μg per day as commensurate with 'paediatric' masculinity.

Recently Kuttenn and Mauvais-Jarvis (1975) reported values of urinary excretion of testosterone glucuronoside and urinary excretion of 5α-diol in normal boys and adults and several patients with abnormal sex development. They found much lower levels of urinary 5α-diol excretion in four prepubertal boys (6-26 μg per 24 hours) than in three postpubertal patients (93-138 μg per 24 hours).

Very recent work in adults by Doberne and New (1976) reported lower levels of urinary excretion of 5α-diol than those of Mauvais-Jarvis et al. (1973). Although they were unable to support the hypothesis that the difference between urinary testosterone and urinary 5α-diol excretion represents the amount of extra-hepatic 5α-reduction of testosterone, they affirmed their use in the clinical evaluation of patients.

Robel et al. (1966a, 1966b) have shown that most of the urinary excretion of 5β-androstane-3α,17β-diol (5β-diol) is formed from the metabolism of testosterone glucuronoside whilst only a minor fraction arises from the metabolism of free (unconjugated) testosterone. Furthermore, as the urinary excretion of 5β-diol exceeds that of 5α-diol (Berthou et al., 1971), the determination of the urinary 5β-diol excretion may be of clinical significance.

Having investigated a large series of male patients with gonadal problems, it was possible to associate the urinary excretion of 5β-diol with the development of the external genitalia, good development of the

penis accompanying high urinary excretion of 5β-diol. By reference to the clinical observations in the patients investigated, and the results of Berthou et al. (1971) on normal males and females, a minimum daily urinary 5β-diol excretion of 50 μg per 24 hours was found to be commensurate with normal genital development.

The urinary excretion of epiandrosterone (EpiA) was found to be raised in several patients in this investigation, with primary testicular failure and inadequate testosterone production. DHA was postulated as the principal anabolic hormone in such patients with inadequate testosterone production and a direct metabolism of DHA to EpiA was suggested from studies of androgen metabolism. Raised urinary excretion of EpiA, then, may indicate a somatic defect secondary either to testosterone lack or tissue insensitivity to testosterone. In these circumstances, the soma cannot utilize testosterone and growth and development are dependent on the weaker androgen, DHA (Hamilton, 1974a). When testosterone is utilized as the anabolic hormone, the urinary excretion of EpiA is low. The urinary excretion of EpiA may therefore give an indication of somatic status of the patient.

The urinary metabolites of testosterone may then have clinical significance as indicators of androgenicity, genital development and somatic utilization of testosterone. The urinary excretion of these metabolites has been found of clinical use, especially when compared to the urinary and plasma testosterone levels, in assessing the pubertal development of many patients with gonadal disorders.

Abnormal gonadotrophin levels, raised plasma testosterone levels and tissue insensitivity to testosterone have recently been reported in the complete form of the testicular feminization syndrome (Judd et al., 1972). This syndrome is characterized clinically by a female phenotype with normal feminization, absent Müllerian derivatives and sparse or absent

sexual hair in association with the 46,XY karyotype and cryptorchidism. Since the syndrome is manifest despite normal (Southren et al., 1965; French et al., 1966; Rivarola et al., 1967) or elevated (Judd et al., 1972) levels of plasma testosterone, then the aetiology of this syndrome lies in the utilization of androgens. Despite the normal or raised levels of plasma testosterone these patients have normal female psycho-sexual differentiation and expression.

Psycho-sexual differentiation is believed to be controlled by the androgens and oestrogens which act on the anterior hypothalamus to determine behaviour, create sexual differentiation of the brain and control gonadotrophin secretion. Sexual desire is no doubt also involved. Although there is strong support for both androgen and oestrogen neurones, Stumpf (1970) postulated that the androgen neurones are in fact oestrogen neurones which will respond to androgens when these are present in sufficient amounts. Later work by Naftolin et al. (1971, 1972) in mature rats and human foetuses demonstrated that in both sexes androgens (testosterone and androstenedione) are aromatized to oestrogens by receptor cells in the hypothalamus. It is the oestrogen so formed that is now thought to determine sexual identity (Naftolin and Ryan, 1975).

In the testicular feminization syndrome the characteristically high gonadotrophin levels can be reduced by testosterone (presumably following conversion to oestrogens) and this is the only androgen effect that can be elicited in this syndrome. Thus in the testicular feminization syndrome although systemic sensitivity to testosterone is lost, the diencephalic cells retain their capacity to convert androgens to oestrogens. It is quite clear from this experiment of nature that the ability of the tissue to utilize testosterone is significant not only in determining the body form but also in determining the psycho-sexual differentiation and what the patient thinks of 'her' body form. Therefore in what follows I will

relate the metabolism of testosterone to the physical form of the patients and attempt to deduce whether their problem is primarily one of testosterone lack or of primary tissue insensitivity or an amalgamation of both. I will leave the concept of hormone-induced psycho-sexual identity to the interested reader.

TABLE 4.I CLINICAL DETAILS OF FOUR PATIENTS WITH THE TESTICULAR FEMINIZATION SYNDROME

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	Weight Percentile
1	0.4	0.4	50	50
2	2.8	3.0	50-75	50-75
3	3.3	2.0	10	10
4	10.1	10.0	97	75

In Table 4.I are shown the clinical data of four patients with the testicular feminization syndrome. Their ages ranged from 5 months to 10.1 years at the time of investigation. It is of interest that three patients, protocols 1, 2 and 4, show a high normal basal plasma testosterone concentration with an extremely good rise by the third day of HCG administration. Singularly patient 3 has a normal low basal plasma testosterone concentration but there is no rise following HCG. It must therefore be argued that this patient has in addition primary testicular failure. Further evidence for this is shown in the urinary testosterone results and retarded bone age for this patient. It can therefore be assumed that while osseous maturation is dependent on androgens in normal males, in these patients with the testicular feminization syndrome bone maturation is dependent on circulating oestrogen (Rosenfield et al., 1971).

These patients with testicular feminization are taken as the prime example of tissue insensitivity and form the basis of the argument that a

study of the urinary androgen metabolites, as represented in the androstanediols, is of significance in determining the tissue metabolism of testosterone.

Clinically patients 1 and 4 are examples of the complete form of the syndrome, their external genitalia being exactly those of a normal female. Patients 2 and 3 have been interpreted as clinical examples of the partially expressed syndrome, as both have a small but significant degree of clitoral enlargement and have presumably partial insensitivity to testosterone (Rosenfield et al., 1971; Madden et al., 1975).

TABLE 4.II EXCRETION OF 5α-ANDROSTANE-3α,17β-DIOL AND 5β-ANDROSTANE-3α,17β-DIOL IN FOUR PATIENTS WITH TESTICULAR FEMINIZATION

Protocol	Age (Years)	5α-diol (μg/24 hrs)		5β-diol (μg/24 hrs)	
		Basal	Day 3	Basal	Day 3
1	0.4	12	26	16	59
2	2.8	11	56	44	402
3	3.3	1	80	109	254
4	10.1	9	17	12	20

It will be noted from Table 4.II that the basal urinary excretion of 5α-diol is low in all patients. This is in agreement with the results of Kuttenn and Mauvais-Jarvis (1975) for prepubertal patients. Following stimulation with HCG the urinary excretion of 5α-diol remains low (< 30 μg per 24 hours; Mauvais-Jarvis et al., 1973) in patients 1 and 4 and a poor androgenic status must be concluded. These patients will thus lack the rugged body form of the true male. Conversely, patients 2 and 3 have a good rise in the urinary excretion of 5α-diol following HCG and therefore if untreated these patients will masculinize at puberty. Kuttenn and Mauvais-Jarvis (1975) have recently found high levels of 5α-diol excretion in adult patients with partial testicular feminization.

From the data on the urinary excretion of 5β-diol the four patients again divide themselves into the two groups. Patients 1 and 4, with the complete form of the syndrome, have a low basal urinary excretion of 5β-diol which remains low following HCG. However, patients 2 and 3, with the incomplete form of testicular feminization, have much higher basal urinary excretion of 5β-diol which rises to very high levels following HCG. If 5β-diol excretion is indeed an index of the genital status then further clitoral enlargement will occur in patients 2 and 3 at puberty.

Compare these results with those from a normal pubertal patient (protocol 66, Chapter 5). This boy, aged 15.6 years at investigation, was of normal height and weight for age and had a bone age (15.5 years) equivalent to his chronological age. He had a normal basal level of plasma testosterone and a good response to administered HCG. The basal urinary excretion of testosterone was also normal for age and again stimulated normally following administered HCG. Of interest and significance is the high basal urinary excretion of 5α-diol (230 µg per 24 hours) which remains high throughout stimulation (Kuttenn and Mauvais-Jarvis, 1975). It will also be noted that the urinary excretion of 5β-diol is at a high basal level and remains high following HCG. These results are in keeping with a normal pubertal boy with normal genital development.

Now observe the urinary excretion of epiandrosterone in this patient (protocol 66, Table 4.III). Here it will be noted that the basal excretion

TABLE 4.III URINARY EXCRETION OF EPIANDROSTERONE AND EFFECT OF HCG

Protocol	Age (Years)	Epiandrosterone (µg/24 hrs)	
		Basal	Day 3
1	0.4	129	356
2	2.8	78	1,163
3	3.3	120	124
4	10.1	2	6
66	15.6	24	13

of epiandrosterone is at a normal low level (Uozumi et al., 1969) and remains low following HCG. This is in agreement with normal testosterone metabolism. Compare this with the urinary excretion of epiandrosterone in patients 1, 2 and 3 with testicular feminization. These patients have an abnormally high basal urinary excretion of epiandrosterone which remains high following HCG. Abnormal metabolism of androgens must be concluded, DHA being the main anabolic hormone. The extremely high value for patient 2 following HCG is then probably due to a partial deficient activity of 17β-reductase. Such a defect has been postulated in other patients with incomplete testicular feminization (Neher and Kahnt, 1966; Saez et al., 1971).

These two groups represent extremes in the spectrum of patients investigated. One is a normal male with the 46,XY karyotype with normal testicular function, whilst the other group have a female phenotype with the 46,XY karyotype due to the testicular feminization syndrome. While generally having good testicular function which is capable of responding to an HCG stimulus, this group differ from the normal male particularly in respect of the urinary excretion of 5α-diol, 5β-diol and epiandrosterone. It is the urinary excretion of these three metabolites that, I postulate, reflects the difference in tissue sensitivity to testosterone. Thus patients with a high urinary excretion of 5α-diol ($>$ 30 µg per 24 hours) have a good androgenic status, patients with a high urinary excretion of 5β-diol ($>$ 50 µg per 24 hours) have a good genital status and patients with a low urinary excretion of epiandrosterone ($<$ 50 µg per 24 hours) have a good somatic status.

Within the remaining patients investigated and now to be discussed I will point out patients who have poor testicular function (in that their plasma testosterone levels are low and do not rise significantly following HCG) and those who have normal levels of plasma testosterone but show altered excretion of the urinary androgen metabolites. The significance of these variants will be discussed.

Consider now the effect of an extra X chromosome in a male karyotype. Two patients are presented here for study, one a typical case of Klinefelter's syndrome with a 47,XXY karyotype, and a patient who has the XX-male syndrome but on whose X chromosome Y chromosome material has been detected in the testes by Dr Ferguson-Smith (personal communication) and will thus be considered a variant of Klinefelter's syndrome. These patients will then be considered as being modified in their testosterone effects somatically by the influence of the extra X chromosome, while their testicular function will be regarded in the light of the known pathology of the Klinefelter testis. Clinical data for these two patients are presented below (Table 4.IV).

TABLE 4.IV CLINICAL DATA OF PATIENTS WITH KLINEFELTER'S SYNDROME

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	Karyotype	Pubertal Stage*
8	8.7	8.3	90	47,XXY	P1 G1
9	14.0	14.0	25	46,XX	P3 G4

* Tanner (1962)

It is clear from the biochemical data presented for these two boys (protocols 8 and 9, Chapter 5) that both have a low normal plasma testosterone level which is satisfactorily increased following HCG. The good response to HCG is also reflected in an appropriate increase in the urinary excretion of testosterone from very low basal levels. These results are in agreement with those of Paulsen et al. (1968), Gabrilove et al. (1970) and Stewart-Bentley and Horton (1973). Therefore while it is the rule that patients with Klinefelter's syndrome undergo testicular atrophy (Lipsett et al., 1966; Hudson et al., 1967; Stewart-Bentley and Horton, 1973; Ionescu et al., 1974), at the ages of these boys when investigated the testicular capacity to produce testosterone was not significantly impaired (Franchimont et al., 1974).

The basal urinary excretion of the 11-deoxy-17-oxosteroids was generally significantly below normal and in particular these patients had a very low basal urinary excretion of aetiocholanolone which is in agreement with the results of Johnsen (1956). The response of these individual 17-oxosteroids to administered HCG was, however, significantly above normal, normal levels for age being reached by the second day of stimulation.

Observe, however, the considerable change in the urinary androgen metabolites which has occurred as the result of the extra X chromosome. Significantly there is low or undetectable urinary excretion of 5α-diol in both patients reflecting the poor androgenicity of these patients. In the younger patient the urinary excretion of 5β-diol is low, this in agreement with his hypogonadism, whilst in the older patient there is normal excretion of 5β-diol which correlates well with the clinical features for he has adequate penis and scrotum although his pubic hair is of the female distribution. In the younger patient (protocol 8, XXY) the urinary excretion of epiandrosterone is normally low and therefore he has normal somatic response to circulating testosterone and is likely to grow to normal or above average proportions. It will be seen that already he is on the 90th percentile for height and the fact that his bone age is retarded indicates that his period of linear growth is longer than his chronological age would indicate and therefore he is likely to grow the taller. The older patient (protocol 9, XX) has an extremely high excretion of epiandrosterone which indicates he cannot utilize testosterone as the main anabolic hormone and he is using the weaker androgen, DHA. This correlates well with his clinical status, his height is retarded, he is of poor physical build and he has marked gynaecomastia. As the basal urinary excretion of 3β-diol was also greatly raised and the response of these compounds to administered HCG was significantly above normal a deficiency of 3β-hydroxysteroid dehydrogenase is indicated.

De la Chapelle (1972), in a review of the XX-male syndrome, reported that the incidence of gynaecomastia in patients with this syndrome (32 per cent) is similar to that found in patients with Klinefelter's syndrome (40 per cent). The patient investigated here had marked gynaecomastia and as a similar enzyme defect was found in a younger patient with slight gynaecomastia (protocol 53, Chapter 5) it was postulated that 3β-hydroxysteroid dehydrogenase deficiency may be a feature of patients with gynaecomastia. However, as complete or partial deficiencies of adrenal 3β-hydroxysteroid dehydrogenase are characteristic of congenital adrenal hyperplasia with the salt-losing syndrome, ambiguous genitalia and often early death (Jänne et al., 1970), it was assumed that the enzyme deficiency postulated in the two patients in this investigation may be limited to the gynaecomastic tissue only. Recently Miller et al. (1974) have confirmed this finding by in vitro incubation studies of gynaecomastia tissue obtained from six patients at mastectomy.

Subsequent analysis of the urinary androgen metabolites of the patient with severe gynaecomastia following ACTH stimulation (protocol 80) shows a very good rise in androsterone and aetiocholanolone in the correct proportions, but there is no significant increase in epiandrosterone excretion. This indicates that the defect is not of adrenal but is likely to be of testicular origin. Recently Jänne et al. (1974) and Rosenfield et al. (1974) have both reported a testicular defect in 3β-hydroxysteroid dehydrogenase in pubertal patients with congenital adrenal hyperplasia and gynaecomastia.

XO/XY mosaicism in the female is characterized by poorly developed secondary sexual characteristics, with evidence of virilization (enlarged clitoris, hypertrichosis and masculine voice), a unilateral testis with a contralateral streak gonad, unilateral or bilateral Müllerian duct derivatives (fallopian tube, uterus) and often stigmata of Turner's syndrome. The clinical data on two patients with the XO/XY syndrome are given in Table 4.V.

TABLE 4.V CLINICAL DATA IN PATIENTS WITH THE XO/XY SYNDROME

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	Clitoral Enlargement	Testicular Histology
6	12.3	12.5	10	++	Adenomatous clumped Leydig cells. Sertoli only.
7	14.8	15.2	25	+++	Adenomatous clumped Leydig cells. Hyalinized tubules.

It will be noted that both patients have normal basal plasma testosterone levels with adequate rises following HCG stimulation. This rise is reflected, somewhat unimpressively, in the urinary excretion of testosterone. This correlates well with the histological finding of a single testis with large clumps of Leydig cells in these patients. It is in accord also clinically with the enlarged clitoris noted in these patients. Therefore androgen-dependent tissues here are responding to the increase in testosterone levels, the basal levels being in the male range.

When the urinary androgen metabolites are considered it will be seen that the urinary excretion of 5α-diol is low and this is against a true androgenic status. It is clear that these patients represent an amalgam of the male and female phenotypes. Since basically they are examples of Turner variants it is readily appreciated that their androgenic status is inadequate. The urinary excretion of 5β-diol is high in both patients and this is in keeping with their masculine type of phallic enlargement. In patient 6 the urinary excretion of epiandrosterone is normally low and therefore one can anticipate that as this patient grows older she is likely to be taller than the usual short stature of the typical Turner syndrome. Conversely, patient 7 has a very high level of epiandrosterone excretion and this is against a good somatic status. This patient has almost reached her final adult stature (143 cm at investigation) and she is clearly then of the typical Turner type.

Protocol 5 is a true hermaphrodite with XX/XY mosaicism. At the age of 6.0 years her bone age was 5.8 years and her height on the 50th percentile. The clinical stigmata were those of an enlarged organ with a single perineal opening. The basal urinary excretion of all androgen metabolites was extremely low and the administered HCG in general had no effect although the results were variable. These results are again not compatible with normal testicular function although the increase in plasma testosterone following HCG suggests some, although limited, Leydig cell function. It is no surprise therefore to find that this patient had bilateral ovotestes (Figure 1.7, page 42) the testicular component of which resembled primitive testicular tubules. This patient was somatically more female than male and is being raised successfully in the female sex.

The remaining patients will be classified mainly by their biochemical data. In Table 4.VI are the clinical data from eight patients who have

TABLE 4.VI CLINICAL DATA OF PATIENTS OF SHORT STATURE DUE TO DEFICIENT hGH

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	hGH	LH	Pubertal Stage
10	8.1	6.4	< 3	-	-	G0 P0
11	8.5	5.4	< 3	-	+	G1 P1
12	10.1	7.5	< 3	-	-	G1 P1
13	11.0	7.3	< 3	-	-	G1 P1
14	11.5	8.2	< 3	-	-	G1 P1
15 (Retest of 12)	12.2	8.6	< 3	-	-	G1 P1
16	14.3	9.4	< 3	-	-	G1 P1
17	15.0	12.4	< 3	-	-	G2 P2

been demonstrated to be deficient in human growth hormone (hGH) reserve following insulin hypoglycaemia. These patients have been accepted at the clinical level as being hGH deficient and suitable for hGH administration. It will be seen that all, with the exception of patient 11, are in addition

deficient in luteinizing hormone (LH) production as judged from an LHRH stimulation test.

All patients show normal basal levels of plasma testosterone with generally a small increase following HCG. These increases in plasma testosterone levels were reflected in the urinary excretion of testosterone. The slight increase in testosterone production inferred following HCG is due probably to the quiescent testicular state in the absence of endogenous LH stimulation. As many of these patients had deficient FSH production this is in agreement with the results of Odell et al. (1973) that FSH induces sensitivity to LH. One patient (protocol 10) indeed showed no rise in the plasma testosterone concentration following HCG although the basal level was raised.

All patients show very low urinary excretion of 5α-diol (< 8 µg per 24 hours) and poor androgenicity must be concluded. Three patients (protocols 14, 16 and 17) have in addition a low excretion of 5β-diol, this indicating a poor genital status and supported by the finding of small infantile penis and very small testes (< 3 ml volume) in these patients. It must be concluded that apart from having a poor testicular potential (as LH is absent) these patients show evidence that the external genitalia do not metabolize testosterone normally. These three patients will, with the continued absence, show poor androgenic and genital development probably ultimately being classed as hypogonadal males.

Four patients (protocols 10, 11, 13 and 15), while showing a very low urinary excretion of 5α-diol, have a good urinary excretion of 5β-diol. These four patients also have small external genitalia and testes, despite a good genital status, inferring that given appropriate LH stimulation the prognosis for adequate genital development is good.

All the patients in this group have a low urinary excretion of epiandrosterone, this signifying a good somatic status. Thus if given

adequate replacement hGH therapy, and if the patients can attain adequate levels of endogenous testosterone, then good linear growth is assured.

It has been suggested by some that both testosterone and hGH are necessary for the normal male adolescent growth spurt (Zachmann and Prader, 1970). Moreover, these authors consider hGH may even be necessary to produce normal development of the secondary sex characteristics, although this mechanism of action remains obscure (Zachmann, 1972). The synergistic action of testosterone and hGH on growth may be simply additive (Tanner et al., 1971) or even multiplicative (Prader et al., 1964).

The findings in this investigation could support such a synergistic action of testosterone and hGH on growth, in that patients who do not respond satisfactorily to growth hormone, and have such a pattern of androgen metabolites, would benefit from a therapeutic trial of growth hormone/androgen therapy.

In Table 4.VII are shown the clinical data of eight patients who had no rise of plasma testosterone concentrations following HCG. These patients fall into three distinct clinical and biochemical groups.

TABLE 4.VII CLINICAL DATA OF PATIENTS WITH PRIMARY TESTICULAR FAILURE

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	hGH	Pubertal Stage
18	5.4	4.2	< 3	+	P1 G1
19	6.0	4.3	< 3	+	P1 G1
20	8.3	8.3	10	+	P1 G1
21	10.1	10.1	50	+	P1 G1
22	10.3	9.0	3	+	P1 G1
23	11.8	11.8	10-25	+	P1 G2
24	15.0	12.0	< 3	+	P2 G2
25	15.3	14.2	3	+	P2 G2

The two youngest patients (protocols 18 and 19) are of short stature, with normal levels of hGH and retarded bone age. Both have very small

testes (volumes 0.5 ml and <1 ml respectively). The levels of urinary testosterone excretion and plasma testosterone concentration were at normal levels for age but did not rise following HCG. The urinary excretion of 5α-diol was very low in each patient whilst the urinary excretion of 5β-diol and epiandrosterone were at normal levels. Therefore these patients have a poor androgenic status, but normal genital and somatic latency. Clinically these patients were deduced to have primordial dwarfism with primary testicular failure. Cheek et al. (1966) studied muscle cell size in children with primordial dwarfism and found gross reduction in the number of muscle cells, although individual cell size was normal, and they postulated an in utero failure of adequate cell multiplication. In later work Cheek et al. (1968) found a correlation between total cell population and urinary testosterone glucuronoside excretion, low levels of urinary testosterone being associated with small cell numbers. The primary testicular failure noted in these patients may therefore cause inadequate cell division.

Patients 20, 21 and 23 are taller than the other patients within this group (height percentiles 10-50) and their bone ages are equivalent to their chronological ages. Two of these, patients 21 and 23, are now known to have testicular agenesis and it is possible that the other patient (protocol 20), who presented with bilateral cryptorchidism, may also have testicular agenesis. All have low levels of plasma testosterone with no rise following HCG administration. The urinary excretion of 5α-diol was normally high in all patients as was the urinary excretion of 5β-diol. The urinary excretion of epiandrosterone was normally low in two patients but high in patient 23. It must be concluded that these patients would have reasonable chance of developing into normal adult males were it not for the fact that their testes are incapable of producing testosterone for systemically there is no reason why they cannot utilize testosterone (with the exception of patient 23) if it was present in physiological amounts.

The remaining patients within this set, patients 22, 24 and 25, are of short stature with normal levels of hGH and retarded bone age. All have small testes (< 8 ml volume). The plasma testosterone concentration in these patients is normal or high normal but does not rise following HCG. All patients within this group have a poor androgenic status as judged from the low urinary excretion of 5α-diol, whilst the two older patients have a good genital status inferred from the high urinary excretion of 5β-diol. As these patients have normal levels of LH it must be concluded that the testes in these patients are maximally stimulated and their prognosis for future development is poor.

The remaining patients in this thesis show a variable clinical picture and are thus grouped according to the urinary excretion of 5α-diol, 5β-diol and epiandrosterone. The biochemical findings in these patients will now be discussed and correlated with the clinical data. The prognosis for future development will be given where possible.

The first group to be considered (Group A) have a low urinary excretion of 5α-diol, a low urinary excretion of 5β-diol and a low urinary excretion of epiandrosterone. Their clinical data are given in Table 4.VIII.

It will be noted from this table that five patients (protocols 29, 30, 35, 38 and 39) are of short stature despite normal levels of hGH and normal systemic testosterone utilization. These five patients, however, all have other basic problems in addition to small testes and their short stature is believed to be part of those syndromes. All other patients are within the normal to upper percentiles for height and thus they have no growth problem, in keeping with their good somatic status. Their main problem is small testes, many undescended, in addition to their presenting symptom of microgenitalia.

It is interesting that patients 26, 27 and 28 are brothers with familial inheritance of bilateral undescended testes. The two older

TABLE 4.VIII CLINICAL DATA OF PATIENTS IN GROUP A (GOOD SOMATIC STATUS ONLY)

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	hGH	Pubertal Stage
26	4.1	4.1	75	+	P1 G1 UU*
27	6.0	6.0	75	+	P1 G1 UU
28	7.3	7.3	97	+	P1 G1 UU
29	8.2	8.2	< 3	+	P1 G1
30	10.8	10.5	< 3	+	P1 G1
31	10.8	10.8	50-75	+	P1 G1
32	11.0	10.9	25-50	+	P1 G1 U
33	11.2	11.6	75	+	P1 G0 UU
34	11.9	10.1	25-50	+	P2 G2 UU
35	13.5	9.4	< 3	+	P1 G1 U
36	14.8	13.0	10	+	P2 G1
37	15.0	12.5	50	+	P2 G1
38	16.1	12.0	< 3	+	P1 G2
39	19.0	16.0	< 3	+	P4 G4 Following HCG/HMG

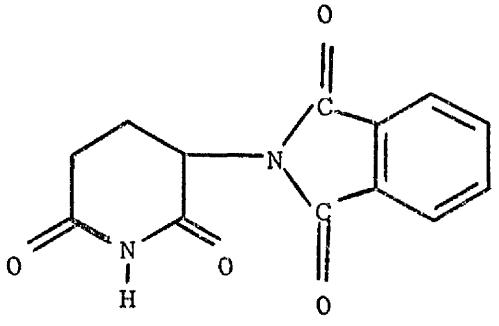
* UU = bilateral undescended testes; U = unilateral undescended testis

brothers had undergone successful bilateral orchidopexy before investigation, whilst the younger brother (protocol 26) presented with bilateral undescended testes, hypospadias and a very immature divided foreskin. Despite successful orchidopexy in the two older brothers these three patients show very similar urinary excretion of androgen metabolites, steroids of both adrenal and testicular origin being very low. Recently Walsh *et al.* (1976) have found similar results for plasma testosterone in patients with familial bilateral undescended testes.

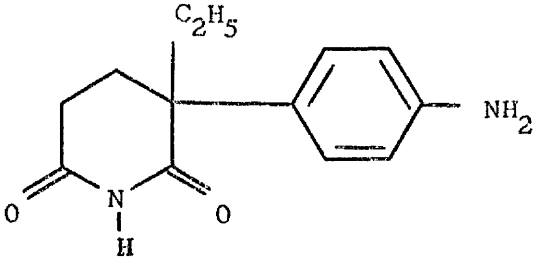
In view of the fact that all patients within this group have normal basal plasma testosterone concentrations and adequate response following HCG stimulation, a normal hypothalamo-pituitary-gonadal axis must be inferred. However, LHRH tests performed on the two eldest patients (protocols 38 and 39) showed impaired response to LHRH indicating that endogenous LH could not maintain plasma testosterone in the post-pubertal age group.

It is interesting that the bone age is equal to the chronological age below 12 years of age but in older patients there is significant retardation in bone maturation. These results are in agreement with my postulate of normal LH levels in the younger patients within this group and progressive failure with age.

Patient 34 has phocomelia, affecting his upper limbs, in consequence to treatment of his mother during the first trimester of pregnancy with thalidomide (α-phthalidoglutarimide) the well-known teratogenic drug originally used as a sedative to alleviate the symptoms of morning sickness.



THALIDOMIDE



AMINOGLUTETHIMIDE

Thalidomide is very similar in structure to aminoglutethimide and both these drugs share the same side-effects, with the exception of teratogenicity. Aminoglutethimide, an anticonvulsant, also causes adrenal inhibition of glucocorticosteroid and mineralocorticosteroid synthesis by inhibition of 20α-hydroxylase in the side-chain cleavage of cholesterol, and is often used in the treatment of adrenal hyperplasia. It is possible that thalidomide may also cause inhibition of 20α-hydroxylase and if this is so this may explain the bilateral testicular undescent and severe hypogonadism noted in this patient.

In conclusion, therefore, this group show poor androgenic status, poor genital status but generally grow well. They may also show evidence of LH deficiency in older patients, being under the third percentile for height as they do not have a pubertal growth spurt.

The second group for consideration (Group B) have a low urinary excretion of 5α-diol, a high urinary excretion of 5β-diol and a low urinary excretion of epiandrosterone and therefore have, by inference, good genital and somatic latency. Their clinical data are shown in Table 4.IX.

TABLE 4.IX CLINICAL DATA OF PATIENTS IN GROUP B (POOR ANDROGENIC STATUS ONLY)

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	hGH	Mean Parental Height Percentile	Pubertal Stage
40	4.1	3.5	3	+		P1 G1
41	4.2	2.4	< 3	+	Congenital scholiosis	P1 G1
42	13.0	11.5	10-25	+	10	P1 G1
43	14.0	11.8	< 3	+	3	P1 G2
44	14.0	10.0	3	+	10 Cushing's	P2 G2
45	16.0	16.0	< 3	+	10	P1 G2
46	16.0	14.0	< 3	+	3	P2 G2
47	16.0	15.0	< 3	+		P2 G2
48	16.0	14.0	10	+	Cyclophosphamide treated	P2 G2
49	16.1	14.5	< 3	+		P2 G2
50 Repeat of 45	17.1	17.2	3	+		P3 G4

While patients of group A were generally of above average height, the patients in this group are all of short stature, despite normal levels of hGH and good somatic latency. One might reasonably have expected these patients to have been taller, but there were good physical or genetic reasons for these patients to be of short stature. Patient 41 had congenital scholiosis while patient 42 had severe anoxia during the third uterine month and in addition the mean parental height was low. Four other patients were of small genetic origin (patients 43-46) whilst another patient had the nephrotic syndrome and had received cyclophosphamide as therapy. Cyclophosphamide (1-bis-(2 chloroethyl)amino-1-oxo-2-azo-6 oxaphosphoridine) is a

heterocyclic mustard used as a cytostatic drug and is known to cause inhibition of spermatogenesis although it is not believed to affect the interstitial cells (Kumar et al., 1972; Vilar, 1974). It is of interest, in light of the repeat test on patient 45 (protocols 50 and 79), that although when first tested at 16 years of age he was under the third percentile for height, one year later he is only on the third percentile and bone age is equal to chronological age. Thus patients in this group are likely to be small if their genetic pattern is small despite a good somatic latency.

All patients within this group presented with retarded gonadal development despite good genital latency as inferred from the high urinary excretion of 5β-diol. This discrepancy may be related to the low urinary excretion of 5α-diol in these patients. Vermeulen et al. (1972) observed a good correlation between the urinary excretion of 5α-diol and the apparent free testosterone concentration. Therefore these patients may have increased binding of testosterone, as noted in other patients with hypogonadism (Vermeulen and Verdonck, 1972). Under these conditions there is an increased conversion of testosterone to 5β-metabolites (Vermeulen and Verdonck, 1972).

Alternatively these patients may have a partial deficiency of 5α-reductase as several patients (41, 43 and 47) show abnormal excretion of androsterone and aetiocholanolone whilst all show diminished urinary excretion of androsterone.

There is evidence that these patients will enter into a late puberty since the retest of patient 45 (protocol 50) showed raised gonadotrophin levels and raised adrenal function, whilst patient 49 has since entered into a seemingly normal but delayed puberty.

The third group for consideration (Group C) have a low urinary excretion of 5α-diol, a high urinary excretion of 5β-diol and a high urinary excretion of epiandrosterone and therefore by inference have good genital latency. Their clinical data are shown in Table 4.X.

TABLE 4.X CLINICAL DATA OF PATIENTS IN GROUP C (GOOD GENITAL STATUS ONLY)

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	Weight Percentile	Pubertal Stage
51	3.8	3.8	50	75	P1 G1
52	6.8	6.8	10	25-50	P1 G1
53	10.9	10.9	75	90	P1 G1
54	11.0	12.5	90	97	P2 G2
55	12.5	12.7	50	90	P2 G2
56	13.7	13.9	50	75-90	P2 G2
57	13.9	13.5	<3	10	P1 G2

There it will be noted that these patients are of normal bone age and genital development. However these patients generally show obesity, this in combination with normal or above normal height. It is interesting that the short stature of patient 57 is of genetic origin.

This group of patients all have good levels of plasma testosterone with normal rises following HCG and thus normal testicular function must be inferred. Similarly normal adrenal function must be concluded from the normal urinary excretion of individual 17-oxosteroids noted in these patients. Their problem is thus one of metabolism and is in keeping with the high urinary excretion of epiandrosterone noted in these patients. It was inferred that these patients were of poor somatic latency, yet all grow well and it must be concluded that other factors are causing raised urinary epiandrosterone in these patients. It is interesting that in patients 51, 52 and 55 the urinary excretion of epiandrosterone falls following HCG whilst in patient 53, with gynaecomastia, the grossly elevated epiandrosterone excretion is probably due to a partial 3β-hydroxysteroid dehydrogenase defect as postulated earlier (page 225).

The next group for consideration (Group D) have a low urinary excretion of 5α-diol, a low urinary excretion of 5β-diol and a raised urinary

excretion of epiandrosterone and therefore have poor potential. Their clinical data are shown in Table 4.XI.

TABLE 4.XI CLINICAL DATA OF PATIENTS IN GROUP D (POOR POTENTIAL)

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	Undescended Testes	Pubertal Stage
58	4.5	6.5	25-50	UU	P1 G0
59	5.4	6.0	10-25	UU	P1 G0
60	9.5	-	50	UU	P1 G0
61	9.6	11.0	97	UU	P1 G1
62	10.0	-	3	UU	P1 G1
63	10.9	8.0	50	UU	P1 G0
64	14.4	12.1	<3	-	P1 G1

There it will be seen that although these patients were of variable height and had varying degrees of bone maturation nearly all of them show bilateral undescended testes and severe hypogonadism. The majority of these patients have a normal plasma testosterone concentration and good response to HCG and therefore their problem is probably one of metabolism as they have adequate response to HCG (Walsh et al., 1976). It is therefore of interest that these patients with bilateral undescended testes all had a low basal urinary excretion of androsterone, aetiocholanolone and DHA but an elevated urinary excretion of EpiA. The urinary excretion of EpiA is normally much lower than that of the other 11-deoxy-17-oxosteroids (Uozumi et al., 1969) and therefore these results imply abnormal metabolism of the androgens. This abnormal metabolism was confirmed by the elevated A/Ae ratio in most patients. The undetectable or significantly subnormal urinary excretion of both DHA and Δ^5-diol implys a deficiency of 17,20-desmolase in these patients with bilateral undescended testes. The low urinary excretion of both 5α-diol and 5β-diol probably indicates reduced androgenicity (hypogonadism) and not tissue insensitivity

(Mauvais-Jarvis et al., 1968) as both of these metabolites had a poor response to administered HCG.

The final group for discussion (Group E) have good urinary excretion of 5α-diol, normal urinary excretion of 5β-diol and low urinary excretion of epiandrosterone and therefore have normal latency for development. Their clinical data are shown in Table 4.XII. There it will be noted that

TABLE 4.XII CLINICAL DATA OF PATIENTS IN GROUP E (NORMALS)

Protocol	Age (Years)	Bone Age (Years)	Height Percentile	Pubertal Stage
65	15.0	15.0	50	P3 G3
66	15.6	15.5	50	P3 G3
67	20.0	19.5	50	P5 G5
68	26.0	26.0	50	P5 G5

all these patients have normal bone maturation, are of normal height and normal development of secondary sexual characteristics. Their gonadal development and testicular size are in agreement with their normal urinary excretion of androstanediols whilst all, with the exception of patient 65, show normal adrenal function inferred from the urinary excretion of individual 17-oxosteroids. Patient 65 presented with Cushing's syndrome and it is of interest that he showed a raised aetiocholanolone/androsterone ratio typical of this syndrome (James, 1961).

Similar grouping of the remaining patients considered in this thesis produced several very small groups of one to two patients and thus aetiology and prognosis cannot be given. However, all of these patients when considered individually show urinary excretion of androgen metabolites equivalent to their development.

The urinary metabolites of testosterone may then have clinical significance as indicators of androgenicity, genital development and

somatic utilization of testosterone. The urinary excretion of these metabolites has been found clinically suggestive, especially when compared to the urinary and plasma testosterone levels, in assessing the pubertal development of many patients with gonadal disorders.

The method of assessing testicular function, developed and applied in this investigation and described in this thesis, aims at estimating the ability of the testes to produce testosterone and the ability of the soma in general to utilize the available testosterone. It does not resolve the quest for the basic aetiology of many gonadal disorders but it points to several enzyme defects which may contribute to the aetiology of such disorders.

CHAPTER 5

PROTOCOLS

The results from individual patients are tabulated in this Chapter. These results are included merely as an addendum for the reader who may wish to compare individual results within patients.

Patients are identified here by first name and initial of the surname only for ethical reasons. The reader requiring further information is referred to the case records at the relevant hospital of admission.

The following abbreviations are used in the tables:-

ND	Not detectable
NM	Not measured (used in cases where the retention time of a particular steroid differs by more than 0.2 minutes from the retention time of the standard steroid).
P	Pubertal hair stage (Tanner, 1962)
G	Genital stage (Tanner, 1962)

Protocol	1
Name	Allison P.
Hospital of admission	Royal Northern Infirmary, Inverness
Hospital number	047512
Chronological age	0.4 years
Bone age	0.4 years
Height percentile	50
Pubertal stage	P1 G1 (female)
Clinical diagnosis	Testicular feminization syndrome
Karyotype	46,XY

URINARY CREATININE: 48-76 (mean 61) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.20 mg per 24 hours
Day 1	0.27
Day 2	0.21
Day 3	0.12

URINARY TESTOSTERONE

Basal	0.22 µg per 24 hours
Day 1	0.25
Day 2	0.41
Day 3	0.78

PLASMA TESTOSTERONE

Basal	479 ng per 100 ml
Day 3	1,886

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	12	15	14	26
5β-Androstane-3α,17β-diol	16	29	31	59
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	ND	ND	ND	ND
Androsterone	19	9	3	13
Aetiocholanolone	8	8	7	16
Dehydroepiandrosterone	23	58	83	128
Epiandrosterone	129	194	354	356

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	2
Name	Allison Q.
Hospital of admission	RHSC, Glasgow
Hospital number	136443
Chronological age	2.8 years
Bone age	3.2 years
Height percentile	50-75
Pubertal stage	P1 G1 (female)
Clinical diagnosis	Testicular feminization syndrome
Karyotype	46,XY

URINARY CREATININE: 90-206 (mean 149) mg per 24 hours

URINARY 17-OXOSTEROIDS		
	Basal	0.12 mg per 24 hours
	Day 1	0.12
	Day 2	0.23
	Day 3	0.31

URINARY TESTOSTERONE		
	Basal	0.19 µg per 24 hours
	Day 1	NM
	Day 2	1.51
	Day 3	0.96

PLASMA TESTOSTERONE		
	Basal	501 ng per 100 ml
	Day 3	3,446

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	11	12	15	56
5β-Androstane-3α,17β-diol	44	56	128	402
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	113	152	190	562
Androsterone	152	167	160	277
Aetiocholanolone	56	74	105	97
Dehydroepiandrosterone	ND	ND	ND	ND
Epiandrosterone	78	96	121	1,163
11β-Hydroxyandrosterone	1,102	NM	NM	1,159

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	3
Name	Sandra T.
Hospital of admission	RHSC, Glasgow
Hospital number	135192
Chronological age	3.3 years
Bone age	2.0 years
Height percentile	10
Pubertal stage	P1 G1 (female)
Clinical diagnosis	Testicular feminization syndrome
Karyotype	46,XY

URINARY CREATININE: 209-300 (mean 246) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.58 mg per 24 hours
Day 1	0.15
Day 2	0.03
Day 3	0.03

URINARY TESTOSTERONE

Basal	1.12 µg per 24 hours
Day 1	NM
Day 2	1.23
Day 3	1.27

PLASMA TESTOSTERONE

Basal	76 ng per 100 ml
Day 3	77

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	21	37	80
5β-Androstane-3α,17β-diol	109	117	184	254
Δ^5-Androstene-3β,17β-diol	29	58	ND	ND
5α-Androstane-3β,17β-diol	153	243	163	190
Androsterone	133	136	206	287
Aetiocholanolone	16	9	63	41
Dehydroepiandrosterone	80	84	95	139
Epiandrosterone	120	124	124	124

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	4
Name	Allison A.
Hospital of admission	RHSC, Glasgow
Hospital number	122910
Chronological age	10.1 years
Bone age	10.0 years
Height percentile	97
Pubertal stage	P1 G1 (female)
Clinical diagnosis	Testicular feminization syndrome
Karyotype	46,XY

URINARY CREATININE: 512-651 (mean 587) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.58 mg per 24 hours
Day 1	0.58
Day 2	1.18
Day 3	1.35

URINARY TESTOSTERONE

Basal	2.31 µg per 24 hours
Day 1	2.63
Day 2	5.47
Day 3	6.88

PLASMA TESTOSTERONE

Basal	1,079 ng per 100 ml
Day 3	15,219

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	9	37	59	17
5β-Androstane-3α,17β-diol	12	33	24	20
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	30	64	86	53
Androsterone	150	200	223	272
Aetiocholanolone	79	90	115	125
Dehydroepiandrosterone	19	71	148	44
Epiandrosterone	2	19	27	6

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	5
Name	Andrea S.
Hospital of admission	Vienna
Chronological age	6.0 years
Bone age	5.8 years
Height percentile	50
Pubertal stage	P1 G1 (female)
Clinical diagnosis	XX/XY syndrome
Karyotype	46,XX/46,XY

URINARY CREATININE: 211-467 (mean 299) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal 1	0.03 mg per 24 hours
Basal 2	0.05
Day 1	0.24
Day 2	0.53
Day 3	0.32

URINARY TESTOSTERONE

Basal 1	0.18 µg per 24 hours
Basal 2	0.19
Day 1	0.18
Day 2	0.18
Day 3	0.19

PLASMA TESTOSTERONE

Basal	158 ng per 100 ml
Day 3	283

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal 1	Basal 2	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	1	1	1
5β-Androstane-3α,17β-diol	1	3	2	1	1
Δ^5-Androstene-3β,17β-diol	1	1	1	1	1
5α-Androstane-3β,17β-diol	1	1	1	1	1
Androsterone	11	11	13	18	13
Aetiocholanolone	1	1	99	45	12
Dehydroepiandrosterone	1	1	52	31	3
Epiandrosterone	1	1	17	14	1

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	6
Name	Dianne W.
Hospital of admission	Newcastle General Hospital, Childrens Dept, Newcastle upon Tyne
Hospital number	13146
Chronological age	12.3 years
Bone age	12.0 years
Height percentile	10
Pubertal stage	P3 G2 (female)
Clinical diagnosis	XO/XY syndrome[1]
Karyotype	45,XO/46,XY

URINARY CREATININE		356-734 (mean 567) mg per 24 hours
URINARY 17-OXOSTEROIDS	Basal	2.10 mg per 24 hours
	Day 1	3.08
	Day 2	1.33
	Day 3	0.83
URINARY TESTOSTERONE	Basal	0.31 µg per 24 hours
	Day 1	0.34
	Day 2	0.63
	Day 3	0.77
PLASMA TESTOSTERONE	Basal	479 ng per 100 ml
	Day 3	845

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	38	15	21	8
5β-Androstane-3α,17β-diol	50	127	23	46
Δ^5-Androstene-3β,17β-diol	8	8	7	7
5α-Androstane-3β,17β-diol	9	8	7	9
Androsterone	467	650	666	761
Aetiocholanolone	327	483	267	717
Dehydroepiandrosterone	157	77	79	77
Epiandrosterone	8	23	8	19

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	7
Name	Moira McC.
Hospital of admission	RHSC, Glasgow
Hospital number	6320
Chronological age	14.8 years
Bone age	15.2 years
Height percentile	25
Pubertal stage	P3 G3 (female)
Clinical diagnosis	XO/XY syndrome
Karyotype	45,XO/46,XY

URINARY CREATININE		670-987 (mean 821) mg per 24 hours
URINARY 17-OXOSTEROIDS	Basal	1.02 mg per 24 hours
	Day 1	0.88
	Day 2	0.85
URINARY TESTOSTERONE	Basal	2.12 µg per 24 hours
	Day 1	-
	Day 2	2.59
PLASMA TESTOSTERONE	Basal	225 ng per 100 ml
	Day 2	711

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2
5α-Androstane-3α,17β-diol	29	28	21
5β-Androstane-3α,17β-diol	140	131	112
Δ^5-Androstene-3β,17β-diol	ND	ND	ND
5α-Androstane-3β,17β-diol	ND	ND	ND
Androsterone	418	436	488
Aetiocholanolone	153	161	153
Dehydroepiandrosterone	146	154	172
Epiandrosterone	657	639	623

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1 and

Protocol	8
Name	James B.
Hospital of admission	RHSC, Glasgow
Hospital number	75439
Chronological age	8.7 years
Bone age	8.3 years
Height percentile	90
Pubertal stage	P1 G1
Clinical diagnosis	Klinefelter's syndrome
Karyotype	47,XXY

URINARY CREATININE: 524-659 (mean 588) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.02 mg per 24 hours
Day 1	0.79
Day 2	1.01
Day 3	1.03

URINARY TESTOSTERONE

Basal	0.22 µg per 24 hours
Day 1	0.37
Day 2	0.47
Day 3	0.66

PLASMA TESTOSTERONE

Basal	78 ng per 100 ml
Day 3	289

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	ND	ND	ND	ND
5β-Androstane-3α,17β-diol	37	8	9	13
Δ^5-Androstene-3β,17β-diol	ND	ND	5	22
5α-Androstane-3β,17β-diol	44	15	7	4
Androsterone	52	68	135	235
Aetiocholanolone	7	15	44	76
Dehydroepiandrosterone	11	18	38	59
Epiandrosterone	45	6	9	10

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	9
Name	Alexander G.
Hospital of admission	RHSC, Glasgow
Hospital number	16663
Chronological age	14.0 years
Bone age	14.0 years
Height percentile	10-25
Pubertal stage	P2 G3
Clinical diagnosis	Gynaecomastia, XX-male
Karyotype	46,XX

URINARY CREATININE: 1,034-1,392 (mean 1,191) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.42 mg per 24 hours
Day 1	2.12
Day 2	1.24
Day 3	2.07

URINARY TESTOSTERONE

Basal	3.62 µg per 24 hours
Day 1	6.66
Day 2	8.70
Day 3	9.87

PLASMA TESTOSTERONE

Basal	350 ng per 100 ml
Day 3	1,166

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	4	12
5β-Androstane-3α,17β-diol	51	383	520	81
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	60	54	1,005	NM
Androsterone	268	374	680	751
Aetiocholanolone	70	113	252	269
Dehydroepiandrosterone	128	276	308	332
Epiandrosterone	238	581	3,289	2,469

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	10
Name	Harvey C.
Hospital of admission	RHSC, Glasgow
Hospital number	172158
Chronological age	8.1 years
Bone age	6.4 years
Height percentile	<3
Pubertal stage	G0 P1
Clinical diagnosis	hGH deficient, LH deficient

URINARY CREATININE: 126-353 (mean 270) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.10 mg per 24 hours
Day 1	0.04
Day 2	0.04
Day 3	0.02

URINARY TESTOSTERONE

Basal	1.00 µg per 24 hours
Day 1	0.51
Day 2	1.03
Day 3	1.35

PLASMA TESTOSTERONE

Basal	523 ng per 100 ml
Day 3	515

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	1	3
5β-Androstane-3α,17β-diol	131	78	127	127
Δ^5-Androstene-3β,17β-diol	4	3	4	6
5α-Androstane-3β,17β-diol	65	37	41	31
Androsterone	128	67	77	58
Aetiocholanolone	16	10	17	22
Dehydroepiandrosterone	28	17	13	18
Epiandrosterone	42	22	32	87

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	11
Name	John B.
Hospital of admission	RHSC, Glasgow
Hospital number	185142
Chronological age	8.5 years
Bone age	5.4 years
Height percentile	< 3
Pubertal stage	G1 P1
Clinical diagnosis	hGH deficient

URINARY CREATININE — 196-345 (mean 279) mg per 24 hours

URINARY 17-OXOSTEROIDS		
	Basal	0.06 mg per 24 hours
	Day 1	0.16
	Day 2	0.82
	Day 3	1.01

URINARY TESTOSTERONE		
	Basal	0.74 µg per 24 hours
	Day 1	0.80
	Day 2	1.00
	Day 3	1.17

PLASMA TESTOSTERONE		
	Basal	103 ng per 100 ml
	Day 3	241

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	8	6	12	15
5β-Androstane-3α,17β-diol	90	53	69	72
Δ^5-Androstene-3β,17β-diol	148	63	25	22
5α-Androstane-3β,17β-diol	128	32	27	102
Androsterone	54	31	115	613
Aetiocholanolone	35	25	90	234
Dehydroepiandrosterone	13	17	19	41
Epiandrosterone	10	13	12	20
11β-Hydroxyandrosterone	176	190	387	848
11β-Hydroxyaetiocholanolone	113	129	542	859

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	12
Name	John S.
Hospital of admission	RHSC, Glasgow
Hospital number	153068
Chronological age	10.1 years
Bone age	7.5 years
Height percentile	< 3
Pubertal stage	G1 P1
Clinical diagnosis	hGH deficient, LH deficient

URINARY CREATININE		113-148 (mean 133) mg per 24 hours
URINARY 17-OXOSTEROIDS	Basal	0.27 mg per 24 hours
	Day 1	0.36
	Day 2	0.58
	Day 3	0.70
URINARY TESTOSTERONE	Basal	0.37 µg per 24 hours
	Day 1	0.39
	Day 2	0.68
	Day 3	1.58
PLASMA TESTOSTERONE	Basal	384 ng per 100 ml
	Day 3	535

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	13
Name	Paul C.
Hospital of admission	RHSC, Glasgow
Hospital number	175691
Chronological age	11.0 years
Bone age	7.3 years
Height percentile	< 3
Pubertal stage	P1 G1
Clinical diagnosis	hGH deficient, LH deficient

URINARY CREATININE: 294-509 (mean 385) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.37 mg per 24 hours
Day 1	1.10
Day 2	0.88
Day 3	0.42

URINARY TESTOSTERONE

Basal	1.41 µg per 24 hours
Day 1	1.69
Day 2	1.79
Day 3	1.95

PLASMA TESTOSTERONE

Basal	112 ng per 100 ml
Day 3	238

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	2	1	2
5β-Androstane-3α,17β-diol	91	72	48	34
Δ^5-Androstene-3β,17β-diol	2	18	7	8
5α-Androstane-3β,17β-diol	12	18	10	5
Androsterone	18	15	17	10
Aetiocholanolone	6	33	31	12
Dehydroepiandrosterone	1	2	2	3
Epiandrosterone	11	13	12	10

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	14
Name	Graham McA
Hospital of admission	RHSC, Glasgow
Hospital number	189459
Chronological age	11.5 years
Bone age	8.2 years
Height percentile	< 3
Pubertal stage	P1 G1
Clinical diagnosis	hGH deficient, LH deficient, surgically corrected bilateral undescended testes

URINARY CREATININE: 283-374 (mean 338) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.45 mg per 24 hours
Day 1	0.41
Day 2	0.37
Day 3	0.44

URINARY TESTOSTERONE

Basal	0.16 µg per 24 hours
Day 1	0.07
Day 2	0.17
Day 3	0.26

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	2	2	3	3
5β-Androstane-3α,17β-diol	5	2	4	4
Δ^5-Androstene-3β,17β-diol	7	2	46	5
5α-Androstane-3β,17β-diol	46	61	970	39
Androsterone	4	20	35	57
Aetiocholanolone	2	1	6	30
Dehydroepiandrosterone	2	1	4	4
Epiandrosterone	73	1	6	2

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	15
Name	John S.
Hospital of admission	RHSC, Glasgow
Hospital number	153068
Chronological age	12.2 years
Bone age	8.6 years
Height percentile	< 3
Pubertal stage	P1 G1
Clinical diagnosis	hGH deficient

URINARY CREATININE: 496-787 (mean 664) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.99 mg per 24 hours
Day 1	0.62
Day 2	0.09
Day 3	0.30

URINARY TESTOSTERONE

Basal	1.93 µg per 24 hours
Day 1	1.11
Day 2	6.25
Day 3	7.14

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	4	4	5	5
5β-Androstane-3α,17β-diol	49	47	52	77
Δ^5-Androstene-3β,17β-diol	12	14	13	16
5α-Androstane-3β,17β-diol	3	3	6	9
Androsterone	359	335	362	481
Aetiocholanolone	199	92	157	161
Dehydroepiandrosterone	48	20	53	55
Epiandrosterone	17	4	11	6

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	16
Name	Brian L.
Hospital of admission	RHSC, Glasgow
Hospital number	3596
Chronological age	14.3 years
Bone age	9.4 years
Height percentile	<3
Pubertal stage	P1 G1
Clinical diagnosis	hGH deficient

URINARY CREATININE: 360-679 (mean 578) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.99 mg per 24 hours
Day 1	1.02
Day 2	0.92
Day 3	0.78

URINARY TESTOSTERONE

Basal	3.31 µg per 24 hours
Day 1	3.83
Day 2	5.77
Day 3	7.55

PLASMA TESTOSTERONE

Basal	679 ng per 100 ml
Day 3	853

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	3	4	6
5β-Androstane-3α,17β-diol	39	28	35	40
Δ^5-Androstene-3β,17β-diol	25	7	9	15
5α-Androstane-3β,17β-diol	18	3	4	8
Androsterone	111	113	227	192
Aetiocholanolone	95	103	165	162
Dehydroepiandrosterone	50	52	33	48
Epiandrosterone	15	8	10	10
11β-Hydroxyandrosterone	128	117	125	120
11β-Hydroxyaetiocholanolone	248	98	133	60

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	17
Name	William McM.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	592994/4
Chronological age	15.0 years
Bone age	12.4 years
Height percentile	<3
Pubertal stage	G2 P2
Clinical diagnosis	hGH deficient

URINARY CREATININE: 422-847 (mean 785) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.43 mg per 24 hours
Day 1	0.75
Day 2	1.18
Day 3	1.47

URINARY TESTOSTERONE

Basal	3.37 µg per 24 hours
Day 1	3.75
Day 2	4.83
Day 3	7.57

PLASMA TESTOSTERONE

Basal	476 ng per 100 ml
Day 3	683

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	4	3	3
5β-Androstane-3α,17β-diol	18	19	25	26
Δ^5-Androstene-3β,17β-diol	1	1	6	1
5α-Androstane-3β,17β-diol	1	1	1	2
Androsterone	96	216	339	487
Aetiocholanolone	199	347	489	626
Dehydroepiandrosterone	202	232	283	281
Epiandrosterone	46	28	48	30

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	18
Name	Alexander S.
Hospital of admission	RHSC, Glasgow
Hospital number	160290
Chronological age	5.4 years
Bone age	4.2 years
Height percentile	< 3
Pubertal stage	P1 G1
Clinical diagnosis	Primordial dwarfism

URINARY CREATININE: 88-148 (mean 109) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.19 mg per 24 hours
Day 1	0.10
Day 2	0.11
Day 3	NM

URINARY TESTOSTERONE

Basal	2.99 µg per 24 hours
Day 1	2.29
Day 2	1.03
Day 3	1.13

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	1	1
5β-Androstane-3α,17β-diol	61	99	3	2
Δ^5-Androstene-3β,17β-diol	20	16	35	10
5α-Androstane-3β,17β-diol	9	7	20	122
Androsterone	42	40	59	97
Aetiocholanolone	2	1	1	1
Dehydroepiandrosterone	4	2	2	7
Epiandrosterone	2	2	3	7

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	19
Name	Thomas F.
Hospital of admission	RHSC, Glasgow
Hospital number	133309
Chronological age	6.0 years
Bone age	4.3 years
Height percentile	<3
Pubertal stage	P1 G1
Clinical diagnosis	Primordial dwarfism, bilateral undescended testes

URINARY CREATININE: 51-147 (mean 147) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.38 mg per 24 hours
Day 1	ND
Day 2	ND
Day 3	0.14

URINARY TESTOSTERONE

Basal	4.00 µg per 24 hours
Day 1	2.58
Day 2	2.06
Day 3	1.30

PLASMA TESTOSTERONE

Basal	92 ng per 100 ml
Day 3	74

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	2	1	1	1
5β-Androstane-3α,17β-diol	71	27	19	19
Δ^5-Androstene-3β,17β-diol	5	4	2	1
5α-Androstane-3β,17β-diol	7	8	8	6
Androsterone	28	14	9	8
Aetiocholanolone	19	8	1	1
Dehydroepiandrosterone	4	4	1	2
Epiandrosterone	10	20	7	8

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	20
Name	Alan S.
Hospital of admission	RHSC, Glasgow
Hospital number	129234
Chronological age	8.3 years
Bone age	8.3 years (but slowing)
Height percentile	10
Pubertal stage	P1 G1
Clinical diagnosis	Bilateral undescended testes, ? testicular agenesis

URINARY CREATININE: 358-512 (mean 456) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.38 mg per 24 hours
Day 1	0.70
Day 2	0.63
Day 3	0.64

URINARY TESTOSTERONE

Basal	1.41 µg per 24 hours
Day 1	1.51
Day 2	1.79
Day 3	1.92

PLASMA TESTOSTERONE

Basal	30 ng per 100 ml
Day 3	35

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 3
5α-Androstane-3α,17β-diol	50	76	286
5β-Androstane-3α,17β-diol	128	100	260
Δ^5-Androstene-3β,17β-diol	58	44	266
5α-Androstane-3β,17β-diol	325	320	360
Androsterone	298	259	362
Aetiocholanolone	54	48	73
Dehydroepiandrosterone	70	130	195
Epiandrosterone	47	54	164

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	21
Name	Paul S.
Hospital of admission	RHSC, Glasgow
Hospital number	177539
Chronological age	10.1 years
Bone age	10.1 years (stationary)
Height percentile	50
Pubertal stage	P1 G1
Clinical diagnosis	Testicular agenesis

URINARY CREATININE: 226-355 (mean 354) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.71 mg per 24 hours
Day 1	4.15
Day 2	3.39
Day 3	2.56

URINARY TESTOSTERONE

Basal	6.09 µg per 24 hours
Day 1	6.22
Day 2	4.36
Day 3	7.87

PLASMA TESTOSTERONE

Basal	37 ng per 100 ml
Day 3	67

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	36	26	11	6
5β-Androstane-3α,17β-diol	153	35	156	274
Δ^5-Androstene-3β,17β-diol	4	20	32	19
5α-Androstane-3β,17β-diol	213	17	122	36
Androsterone	126	216	154	115
Aetiocholanolone	82	174	93	360
Dehydroepiandrosterone	17	57	97	255
Epiandrosterone	15	4	8	167

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	22
Name	Colin C.
Hospital of admission	RHSC, Glasgow
Hospital number	276382
Chronological age	10.3 years
Bone age	9.0 years
Height percentile	3
Pubertal stage	P1 G1
Clinical daignosis	Bilateral undescended testes

URINARY CREATININE: 425-660 (mean 616) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.02 mg per 24 hours
Day 1	0.93
Day 2	1.01
Day 3	0.64

URINARY TESTOSTERONE

Basal	3.96 µg per 24 hours
Day 1	4.07
Day 2	4.18
Day 3	4.98

PLASMA TESTOSTERONE

Basal	295 ng per 100 ml
Day 3	309

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	4	3	4	4
5β-Androstane-3α,17β-diol	38	31	19	21
Δ^5-Androstene-3β,17β-diol	2	10	3	2
5α-Androstane-3β,17β-diol	23	16	6	12
Androsterone	146	133	108	161
Aetiocholanolone	66	75	71	103
Dehydroepiandrosterone	36	44	24	9
Epiandrosterone	16	15	11	42

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	23
Name	Frank L.
Hospital of admission	RHSC, Glasgow
Hospital number	121441
Chronological age	11.8 years
Bone age	11.5 years (stationary)
Height percentile	10-25
Pubertal stage	P1 G2
Clinical diagnosis	Testicular agenesis

URINARY TESTOSTERONE	Basal	0.22 µg per 24 hours
	Day 1	0.15
	Day 2	0.06
	Day 3	0.21

PLASMA TESTOSTERONE	Basal	70 ng per 100 ml
	Day 3	39

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
Δ^5-Androstene-3β,17β-diol	16	21	15	6
5α-Androstane-3β,17β-diol	ND	ND	ND	ND
Androsterone	705	661	565	404
Aetiocholanolone	147	194	96	92
Dehydroepiandrosterone	ND	ND	ND	ND
Epiandrosterone	196	186	294	202
11β-Hydroxyandrosterone	332	255	206	278
11β-Hydroxyaetiocholanolone	64	129	93	140

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	24
Name	Donald McK.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	571631/X
Chronological age	15.0 years
Bone age	12.0
Height percentile	< 3
Pubertal stage	P2 G2
Clinical diagnosis	Short stature, hGH normal

URINARY CREATININE: 584-651 (mean 619) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.43 mg per 24 hours
Day 1	1.26
Day 2	1.69
Day 3	2.37

URINARY TESTOSTERONE

Basal	10.33 µg per 24 hours
Day 1	6.06
Day 2	6.03
Day 3	5.98

PLASMA TESTOSTERONE

Basal	327 ng per 100 ml
Day 3	318

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	4	4	3	6
5β-Androstane-3α,17β-diol	48	35	37	60
Δ^5-Androstene-3β,17β-diol	12	3	3	25
5α-Androstane-3β,17β-diol	17	8	4	6
Androsterone	141	132	130	190
Aetiocholanolone	109	80	104	153
Dehydroepiandrosterone	31	4	7	11
Epiandrosterone	100	2	6	9

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	25
Name	Robin T.
Hospital of admission	RHSC, Glasgow
Hospital number	166237
Chronological age	15.3 years
Bone age	14.2 years
Height percentile	<3
Pubertal stage	P2 G2
Clinical diagnosis	Short stature, hGH normal

URINARY CREATININE: 619-809 (mean 740) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.44 mg per 24 hours
Day 1	0.59
Day 2	0.56

URINARY TESTOSTERONE

Basal	12.61 µg per 24 hours
Day 1	4.87
Day 2	8.85

PLASMA TESTOSTERONE

Basal	1,021 ng per 100 ml
Day 2	666

URINARY ANDROGEN METABOLITES

	µg per 24 hours		
	Basal	Day 1	Day 2
5α-Androstane-3α,17β-diol	32	NM	34
5β-Androstane-3α,17β-diol	75	138	99
Δ^5-Androstene-3β,17β-diol	ND	ND	ND
5α-Androstane-3β,17β-diol	ND	ND	ND
Androsterone	135	120	140
Aetiocholanolone	80	70	87
Dehydroepiandrosterone	251	415	371
Epiandrosterone	382	1,216	932

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1 and 2.

Protocol	26
Name	Gary B.
Hospital of admission	RHSC, Glasgow
Hospital number	176616
Chronological age	4.1 years
Bone age	4.1 years
Height percentile	75
Pubertal stage	P1 G1
Clinical diagnosis	Bilateral undescended testes, Hypospadias

URINARY CREATININE: 150-201 (mean 168) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.16 mg per 24 hours
Day 1	0.10
Day 2	0.18
Day 3	0.16

URINARY TESTOSTERONE

Basal	0.53 µg per 24 hours
Day 1	0.60
Day 2	0.74
Day 3	0.81

PLASMA TESTOSTERONE

Basal	75 ng per 100 ml
Day 3	194

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	9	1
5β-Androstane-3α,17β-diol	18	30	48	28
Δ^5-Androstene-3β,17β-diol	ND	1	ND	ND
5α-Androstane-3β,17β-diol	1	1	1	1
Androsterone	16	14	18	39
Aetiocholanolone	1	1	2	4
Dehydroepiandrosterone	3	1	1	4
Epiandrosterone	4	62	11	1
11β-Hydroxyandrosterone	139	140	138	139
11β-Hydroxyaetiocholanolone	37	31	NM	NM

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	27
Name	Michael B.
Hospital of admission	RHSC, Glasgow
Hospital number	176617
Chronological age	6.0 years
Bone age	6.0 years
Height percentile	75
Pubertal stage	P1 G1
Clinical diagnosis	Surgically corrected bilateral undescended testes

URINARY CREATININE: 175-256 (mean 215) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.11 mg per 24 hours
Day 1	0.11
Day 2	0.02
Day 3	0.11

URINARY TESTOSTERONE

Basal	0.94 µg per 24 hours
Day 1	1.04
Day 2	1.07
Day 3	1.12

PLASMA TESTOSTERONE

Basal	109 ng per 100 ml
Day 3	303

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	6	6	2	6
5β-Androstane-3α,17β-diol	14	32	22	4
Δ^5-Androstene-3β,17β-diol	18	2	13	5
5α-Androstane-3β,17β-diol	19	1	14	1
Androsterone	1	6	1	2
Aetiocholanolone	1	1	8	1
Dehydroepiandrosterone	40	1	28	9
Epiandrosterone	50	4	5	4
11β-Hydroxyandrosterone	109	107	111	108

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	28
Name	Stuart B.
Hospital of admission	RHSC, Glasgow
Hospital number	176618
Chronological age	7.3 years
Bone age	7.3 years
Height percentile	97
Pubertal stage	P1 G1
Clinical diagnosis	Surgically corrected bilateral undescended testes

URINARY CREATININE: 231-331 (mean 276) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.23 mg per 24 hours
Day 1	0.34
Day 2	0.15
Day 3	0.09

URINARY TESTOSTERONE

Basal	1.04 µg per 24 hours
Day 1	1.14
Day 2	1.50
Day 3	1.55

PLASMA TESTOSTERONE

Basal	103 ng per 100 ml
Day 3	418

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	2	10	1	1
5β-Androstane-3α,17β-diol	38	82	53	117
Δ^5-Androstene-3β,17β-diol	89	284	115	54
5α-Androstane-3β,17β-diol	NM	NM	NM	NM
Androsterone	9	35	16	30
Aetiocholanolone	4	2	1	1
Dehydroepiandrosterone	1	6	39	41
Epiandrosterone	92	195	62	19
11β-Hydroxyandrosterone	374	370	376	368
11β-Hydroxyaetiocholanolone	31	39	41	72

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	29
Name	John F.
Hospital of admission	RHSC, Glasgow
Hospital number	102110
Chronological age	8.2 years
Bone age	4.5 years
Height percentile	<3
Pubertal stage	P1 G1
Clinical diagnosis	Small stature, hGH normal, alopecia, hypogonadism

URINARY CREATININE: 190-218 (mean 203) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.35 mg per 24 hours
Day 1	0.28
Day 2	0.37
Day 3	0.35

URINARY TESTOSTERONE

Basal	0.06 µg per 24 hours
Day 1	0.07
Day 2	0.08
Day 3	0.11

PLASMA TESTOSTERONE

Basal	16 ng per 100 ml
Day 3	89

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	6	1
5β-Androstane-3α,17β-diol	5	3	5	1
Δ^5-Androstene-3β,17β-diol	1	3	8	1
5α-Androstane-3β,17β-diol	1	2	6	1
Androsterone	7	15	11	16
Aetiocholanolone	4	5	5	2
Dehydroepiandrosterone	1	2	68	5
Epiandrosterone	1	2	41	7

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	30
Name	Douglas A.
Hospital of admission	RHSC, Glasgow
Hospital number	90102
Chronological age	10.8 years
Bone age	10.5 years
Height percentile	< 3
Pubertal stage	P1 G1
Clinical diagnosis	Short stature, hGH normal

URINARY CREATININE: 273-978 (mean 663) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.49 mg per 24 hours
Day 1	1.38
Day 2	1.51
Day 3	1.61

URINARY TESTOSTERONE

Basal	4.16 µg per 24 hours
Day 1	4.56
Day 2	5.97
Day 3	8.97

PLASMA TESTOSTERONE

Basal	208 ng per 100 ml
Day 3	634

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	4	5	20	27
5β-Androstane-3α,17β-diol	24	35	46	55
Δ^5-Androstene-3β,17β-diol	7	2	4	5
5α-Androstane-3β,17β-diol	16	6	10	29
Androsterone	186	208	321	475
Aetiocholanolone	138	135	255	370
Dehydroepiandrosterone	25	25	61	32
Epiandrosterone	2	19	39	4

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	31
Name	Gary F.
Hospital of admission	RHSC, Glasgow
Hospital number	168317
Chronological age	10.8 years
Bone age	10.8 years
Height percentile	50-75
Pubertal stage	P1 G1
Clinical diagnosis	Turner phenotype

URINARY CREATININE: 466-602 (mean 537) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.40 mg per 24 hours
Day 1	0.65
Day 2	0.89
Day 3	0.97

URINARY TESTOSTERONE

Basal	1.83 µg per 24 hours
Day 1	1.83
Day 2	2.43
Day 3	4.34

PLASMA TESTOSTERONE

Basal	380 ng per 100 ml
Day 3	626

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	28	32	15	14
5β-Androstane-3α,17β-diol	23	17	7	2
Δ^5-Androstene-3β,17β-diol	3	10	14	31
5α-Androstane-3β,17β-diol	19	3	4	5
Androsterone	285	299	332	479
Aetiocholanolone	129	124	129	178
Dehydroepiandrosterone	57	44	49	40
Epiandrosterone	12	1	6	3

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	32
Name	Alisdair R.
Hospital of admission	RHSC, Edinburgh
Chronological age	11.0 years
Bone age	10.9 years
Height percentile	25-50
Pubertal stage	P1 G1
Clinical diagnosis	Prader-Willi syndrome, unilateral undescended testis

URINARY CREATININE: 521-684 (mean 613) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.66 mg per 24 hours
Day 1	0.71
Day 2	0.33
Day 3	0.09

URINARY TESTOSTERONE

Basal	4.18 µg per 24 hours
Day 1	4.20
Day 2	4.34
Day 3	4.64

PLASMA TESTOSTERONE

Basal	224 ng per 100 ml
Day 3	675

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	1	1
5β-Androstane-3α,17β-diol	11	8	8	9
Δ^5-Androstene-3β,17β-diol	1	1	1	1
5α-Androstane-3β,17β-diol	1	1	1	1
Androsterone	250	43	49	154
Aetiocholanolone	108	35	26	69
Dehydroepiandrosterone	50	36	18	43
Epiandrosterone	8	25	5	8

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	33
Name	Frederick G.
Hospital of admission	RHSC, Glasgow
Hospital number	52714
Chronological age	11.2 years
Bone age	11.6 years
Height percentile	75
Pubertal stage	P1 G0
Clinical diagnosis	Bilateral undescended testes, mental deficiency

URINARY CREATININE: 110-433 (mean 265) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.19 mg per 24 hours
Day 1	0.20
Day 2	0.21
Day 3	0.29

URINARY TESTOSTERONE

Basal	0.66 µg per 24 hours
Day 1	0.87
Day 2	0.97
Day 3	1.16

PLASMA TESTOSTERONE

Basal	57 ng per 100 ml
Day 3	161

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	8	3	10	4
5β-Androstane-3α,17β-diol	14	7	14	15
Δ^5-Androstene-3β,17β-diol	9	2	3	2
5α-Androstane-3β,17β-diol	7	2	4	4
Androsterone	169	237	193	195
Aetiocholanolone	89	140	71	99
Dehydroepiandrosterone	12	20	9	21
Epiandrosterone	4	1	4	3
11β-Hydroxyandrosterone	593	583	663	584

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	34
Name	David L.
Hospital of admission	RHSC, Glasgow
Hospital number	48753
Chronological age	11.9 years
Bone age	10.1 years
Height percentile	25-50
Pubertal stage	P2 G2
Clinical diagnosis	Phocomelia, bilateral undescended testes

URINARY CREATININE — 244-324 (mean 290) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.02 mg per 24 hours
Day 1	0.64
Day 2	0.48
Day 3	0.62

URINARY TESTOSTERONE

Basal	7.96 µg per 24 hours
Day 1	10.96
Day 2	11.14
Day 3	15.32

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	3	4	4	5
5β-Androstane-3α,17β-diol	12	17	6	80
Δ^5-Androstene-3β,17β-diol	13	15	4	14
5α-Androstane-3β,17β-diol	4	7	51	33
Androsterone	114	90	113	129
Aetiocholanolone	413	430	451	520
Dehydroepiandrosterone	244	232	330	110
Epiandrosterone	1	10	3	26

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	35
Name	David M.
Hospital of admission	RHSC, Glasgow
Hospital number	100998
Chronological age	13.5 years
Bone age	9.4 years
Height percentile	< 3
Pubertal stage	P1 G1
Clinical diagnosis	Short stature, hGH normal, unilateral undescended testis

URINARY CREATININE: 438-732 (mean 594) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.83 mg per 24 hours
Day 1	0.86
Day 2	1.34
Day 3	1.29

PLASMA TESTOSTERONE

Basal	103 ng per 100 ml
Day 3	274

URINARY ANDROGEN METABOLITES

	µg per 24 hours			
	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	1	1
5β-Androstane-3α,17β-diol	35	35	36	38
Δ^5-Androstene-3β,17β-diol	1	9	19	28
5α-Androstane-3β,17β-diol	8	6	6	5
Androsterone	166	163	228	292
Aetiocholanolone	111	106	135	163
Dehydroepiandrosterone	25	20	28	35
Epiandrosterone	7	2	2	1
11β-Hydroxyandrosterone	120	119	121	120
11β-Hydroxyaetiocholanolone	136	117	112	106

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	36
Name	Edward G.
Hospital of admission	RHSC, Glasgow
Hospital number	168779
Chronological age	14.8 years
Bone age	13.0 years
Height percentile	10
Pubertal stage	P2 G1
Clinical diagnosis	Delayed puberty

URINARY CREATININE: 572-742 (mean 669) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.20 mg per 24 hours
Day 1	0.35
Day 2	0.15
Day 3	0.30

URINARY TESTOSTERONE

Basal	1.25 µg per 24 hours
Day 1	2.54
Day 2	3.51
Day 3	4.19

PLASMA TESTOSTERONE

Basal	1,031 ng per 100 ml
Day 3	1,754

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	2	3	1
5β-Androstane-3α,17β-diol	2	13	5	2
Δ^5-Androstene-3β,17β-diol	1	5	17	12
5α-Androstane-3β,17β-diol	1	1	1	1
Androsterone	50	50	100	124
Aetiocholanolone	36	35	84	109
Dehydroepiandrosterone	5	5	12	8
Epiandrosterone	4	3	4	5

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	37
Name	Paul K.
Hospital of admission	Gartnavel General Hospital, Glasgow
Chronological age	15.0 years
Bone age	12.5 years
Height percentile	50
Pubertal stage	P2 G1
Clinical diagnosis	Cushing's syndrome

URINARY CREATININE: 1,146-1,471 (mean 1,260) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.72 mg per 24 hours
Day 1	1.28
Day 2	1.24
Day 3	1.16

URINARY TESTOSTERONE

Basal	39.30 µg per 24 hours
Day 1	-
Day 2	117.65
Day 3	133.92

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	1	1	3
5β-Androstane-3α,17β-diol	41	70	12	32
Δ^5-Androstene-3β,17β-diol	1	10	17	7
5α-Androstane-3β,17β-diol	2	1	5	1
Androsterone	397	502	582	470
Aetiocholanolone	481	524	583	457
Dehydroepiandrosterone	38	39	35	61
Epiandrosterone	15	23	21	39
11β-Hydroxyandrosterone	456	557	602	574

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	38
Name	James S.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	488014
Chronological age	16.1 years
Bone age	12.0 years
Height percentile	<3
Pubertal stage	P1 G2
Clinical diagnosis	Short stature, hGH normal, LH and FSH deficient

URINARY CREATININE: 291-585 (mean 518) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.95 mg per 24 hours
Day 1	1.28
Day 2	1.71
Day 3	0.58

URINARY TESTOSTERONE

Basal	0.59 µg per 24 hours
Day 1	0.61
Day 2	0.61
Day 3	0.70

PLASMA TESTOSTERONE

Basal	309 ng per 100 ml
Day 3	661

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	8	13	21	26
5β-Androstane-3α,17β-diol	26	76	52	14
Δ^5-Androstene-3β,17β-diol	4	1	9	1
5α-Androstane-3β,17β-diol	3	1	4	1
Androsterone	31	43	169	226
Aetiocholanolone	23	31	53	215
Dehydroepiandrosterone	3	18	94	4
Epiandrosterone	16	23	83	6
11β-Hydroxyandrosterone	274	110	258	139

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	39
Name	Gordon M.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	505081
Chronological age	19.0 years
Bone age	16.0 years
Height percentile	< 3
Pubertal stage	P4 G4
Clinical diagnosis	Short stature, hGH normal, LH and FSH deficient

URINARY CREATININE: 594–736 (mean 683) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.25 mg per 24 hours
Day 1	0.21
Day 2	0.60
Day 3	0.48

URINARY TESTOSTERONE

Basal	3.65 µg per 24 hours
Day 1	2.25
Day 2	5.43
Day 3	6.25

PLASMA TESTOSTERONE

Basal	399 ng per 100 ml
Day 3	1,693

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	15	25	25	54
5β-Androstane-3α,17β-diol	44	50	26	45
Δ^5-Androstene-3β,17β-diol	5	2	4	4
5α-Androstane-3β,17β-diol	10	20	9	10
Androsterone	478	500	662	872
Aetiocholanolone	336	347	419	539
Dehydroepiandrosterone	69	45	65	89
Epiandrosterone	6	4	4	6
11β-Hydroxyandrosterone	296	330	314	332
11β-Hydroxyaetiocholanolone	84	153	27	58

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1–3.

Protocol	40
Name	Ishfaq M.
Hospital of admission	RHSC, Edinburgh
Hospital number	175913
Chronological age	4.1 years
Bone age	3.5 years
Height percentile	3
Pubertal stage	P1 G1
Clinical diagnosis	Male Turner phenotype

URINARY CREATININE: 92-107 (mean 106) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.13 mg per 24 hours
Day 1	0.14
Day 2	0.18
Day 3	0.19

PLASMA TESTOSTERONE

Basal	142 ng per 100 ml
Day 3	368

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	9	2	4	5
5β-Androstane-3α,17β-diol	90	84	105	149
Δ^5-Androstene-3β,17β-diol	22	4	3	3
5α-Androstane-3β,17β-diol	76	100	75	96
Androsterone	79	85	86	124
Aetiocholanolone	10	15	14	33
Dehydroepiandrosterone	67	68	68	66
Epiandrosterone	4	19	9	14

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	41
Name	Raymond McV.
Hospital of admission	RHSC, Glasgow
Hospital number	141860
Chronological age	4.2 years
Bone age	2.4 years
Height percentile	< 3
Pubertal stage	P1 G1
Clinical diagnosis	Short stature, hGH normal, scoliosis, mentally retarded

URINARY CREATININE: 19-54 (mean 33) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.15 mg per 24 hours
Day 1	0.11
Day 2	0.08
Day 3	0.05

URINARY TESTOSTERONE

Basal	6.54 µg per 24 hours
Day 1	6.68
Day 2	9.91
Day 3	12.58

PLASMA TESTOSTERONE

Basal	77 ng per 100 ml
Day 3	467

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	1	7	10	73
5β-Androstane-3α,17β-diol	57	204	629	1,424
Δ^5-Androstene-3β,17β-diol	2	3	6	38
5α-Androstane-3β,17β-diol	1	1	1	2
Androsterone	7	13	16	17
Aetiocholanolone	23	6	9	13
Dehydroepiandrosterone	4	1	2	3
Epiandrosterone	1	2	3	6

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	42
Name	Cliffe J.
Hospital of admission	RHSC, Glasgow
Hospital number	127572
Chronological age	12.9 years
Bone age	11.5 years
Height percentile	10-25
Pubertal stage	P1 G1
Clinical diagnosis	Unilateral undescended testis, obesity

URINARY CREATININE: 368-556 (mean 446) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.30 mg per 24 hours
Day 1	0.31
Day 2	0.55
Day 3	0.61

URINARY TESTOSTERONE

Basal	3.96 µg per 24 hours
Day 1	4.06
Day 2	4.12
Day 3	5.12

PLASMA TESTOSTERONE

Basal	334 ng per 100 ml
Day 3	656

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	10	9	12	14
5β-Androstane-3α,17β-diol	132	221	224	325
Δ^5-Androstene-3β,17β-diol	87	18	19	36
5α-Androstane-3β,17β-diol	5	8	13	13
Androsterone	79	381	410	501
Aetiocholanolone	63	237	406	406
Dehydroepiandrosterone	5	5	29	15
Epiandrosterone	2	5	8	13

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	43
Name	Thomas A.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	573695
Chronological age	14.0 years
Bone age	11.8 years
Height percentile	<3
Pubertal stage	P1 G2
Clinical diagnosis	Short stature, hGH normal

URINARY CREATININE: 540-688 (mean 544) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.65 mg per 24 hours
Day 1	1.40
Day 2	0.72
Day 3	0.57

URINARY TESTOSTERONE

Basal	5.24 µg per 24 hours
Day 1	7.75
Day 2	8.11
Day 3	9.54

PLASMA TESTOSTERONE

Basal	276 ng per 100 ml
Day 3	773

URINARY ANDROGEN METABOLITES · µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	7	4	10	20
5β-Androstane-3α,17β-diol	94	45	63	125
Δ^5-Androstene-3β,17β-diol	11	3	4	11
5α-Androstane-3β,17β-diol	4	2	3	5
Androsterone	121	174	247	588
Aetiocholanolone	218	172	235	576
Dehydroepiandrosterone	25	23	28	38
Epiandrosterone	15	2	4	4

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	44
Name	Thomas G.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	5940196
Chronological age	14.0 years
Bone age	10.0 years
Height percentile	3
Pubertal stage	P2 G2
Clinical diagnosis	Cushing's syndrome

URINARY CREATININE		716-816 (mean 770) mg per 24 hours
URINARY 17-OXOSTEROIDS	Basal	2.45 mg per 24 hours
	Day 1	2.90
	Day 2	3.35
	Day 3	3.99
URINARY TESTOSTERONE	Basal	15.37 µg per 24 hours
	Day 1	15.81
	Day 2	16.79
	Day 3	18.28
PLASMA TESTOSTERONE	Basal	322 ng per 100 ml
	Day 3	705

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	14	15	17	40
5β-Androstane-3α,17β-diol	77	53	85	71
Δ^5-Androstene-3β,17β-diol	1	2	4	18
5α-Androstane-3β,17β-diol	2	2	1	4
Androsterone	152	260	440	501
Aetiocholanolone	117	234	365	412
Dehydroepiandrosterone	53	21	30	128
Epiandrosterone	5	2	5	5
11β-Hydroxyandrosterone	430	513	663	492
11β-Hydroxyaetiocholanolone	54	171	349	315

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	45
Name	David A.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	323308
Chronological age	16.0 years
Bone age	16.0 years
Height percentile	<3
Pubertal stage	P1 G2
Clinical diagnosis	Short stature, hGH normal, delayed puberty

URINARY CREATININE: 572-1,013 (mean 788) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.38 mg per 24 hours
Day 1	1.33
Day 2	1.75
Day 3	1.08

URINARY TESTOSTERONE

Basal	0.14 µg per 24 hours
Day 1	0.27
Day 2	0.47
Day 3	0.56

PLASMA TESTOSTERONE

Basal	55 ng per 100 ml
Day 3	198

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	10	8	8	16
5β-Androstane-3α,17β-diol	69	68	48	53
Δ^5-Androstene-3β,17β-diol	2	3	10	4
5α-Androstane-3β,17β-diol	3	3	2	5
Androsterone	74	121	233	260
Aetiocholanolone	61	101	180	252
Dehydroepiandrosterone	5	29	27	145
Epiandrosterone	53	32	11	11
11β-Hydroxyandrosterone	465	368	338	404
11β-Hydroxyaetiocholanolone	66	155	194	128

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	46
Name	John H.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	557664
Chronological age	16.0 years
Bone age	14.0 years
Height percentile	< 3
Pubertal stage	P2 G2
Clinical diagnosis	Short stature, hGH normal

URINARY CREATININE: 570-740 (mean 652) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.14 mg per 24 hours
Day 1	1.78
Day 2	1.55
Day 3	2.26

URINARY TESTOSTERONE

Basal	0.75 µg per 24 hours
Day 1	0.88
Day 2	1.11
Day 3	1.33

PLASMA TESTOSTERONE

Basal	304 ng per 100 ml
Day 3	3,346

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	21	23	28	54
5β-Androstane-3α,17β-diol	103	96	76	93
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	9	10	1	25
Androsterone	113	150	233	567
Aetiocholanolone	58	97	183	533
Dehydroepiandrosterone	32	53	81	272
Epiandrosterone	9	17	29	76
11β-Hydroxyandrosterone	614	612	601	617

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	47
Name	Michael K.
Hospital of admission	Western Infirmary, Glasgow
Chronological age	16.0 years
Bone age	15.0 years
Height percentile	< 3
Pubertal stage	P2 G2
Clinical diagnosis	Short stature, hGH normal

URINARY CREATININE — 431–845 (mean 599) mg per 24 hours

URINARY 17-OXOSTEROIDS		
	Basal	0.91 mg per 24 hours
	Day 1	1.61
	Day 2	4.65
	Day 3	1.56

URINARY TESTOSTERONE		
	Basal	9.29 µg per 24 hours
	Day 1	10.28
	Day 2	12.38
	Day 3	18.98

PLASMA TESTOSTERONE		
	Basal	299 ng per 100 ml
	Day 2	501
	Day 4	841

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	16	5	10	14
5β-Androstane-3α,17β-diol	85	40	160	292
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	15	6	3	9
Androsterone	61	186	191	565
Aetiocholanolone	62	351	386	937
Dehydroepiandrosterone	27	216	100	161
Epiandrosterone	12	42	27	16
11β-Hydroxyandrosterone	1,341	1,153	1,168	1,269
11β-Hydroxyaetiocholanolone	278	438	450	NM

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1–4.

Protocol	48
Name	David T.S.
Hospital of admission	RHSC, Glasgow
Hospital number	29929
Chronological age	16.0 years
Bone age	14.0 years
Height percentile	10
Pubertal stage	P2 G2
Clinical diagnosis	Nephrotic syndrome, Cyclophosphamide treated

URINARY CREATININE: 420-872 (mean 828) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	3.43 mg per 24 hours
Day 1	3.85
Day 2	3.92
Day 3	3.72

URINARY TESTOSTERONE

Basal	3.79 µg per 24 hours
Day 1	4.68
Day 2	6.35
Day 3	6.70

PLASMA TESTOSTERONE

Basal	392 ng per 100 ml
Day 3	540

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	16	44	11	23
5β-Androstane-3α,17β-diol	76	31	33	96
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	4	3	12	2
Androsterone	4	212	83	535
Aetiocholanolone	4	102	28	314
Dehydroepiandrosterone	18	39	6	190
Epiandrosterone	17	16	4	37
11β-Hydroxyandrosterone	449	428	422	427

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	49
Name	John R.
Hospital of admission	Hawkhead Hospital, Paisley
Hospital number	108069
Chronological age	16.1 years
Bone age	14.5 years
Height percentile	<3
Pubertal stage	P2 G2
Clinical diagnosis	Short stature, hGH normal, delayed puberty

URINARY CREATININE: 486-777 (mean 650) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.13 mg per 24 hours
Day 1	0.84
Day 2	0.90
Day 3	0.92

URINARY TESTOSTERONE

Basal	3.02 µg per 24 hours
Day 1	3.25
Day 2	4.08
Day 3	4.94

PLASMA TESTOSTERONE

Basal	372 ng per 100 ml
Day 3	1,417

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	25	88	38	67
5β-Androstane-3α,17β-diol	60	186	76	101
Δ^5-Androstene-3β,17β-diol	6	14	12	10
5α-Androstane-3β,17β-diol	89	54	51	66
Androsterone	559	582	770	916
Aetiocholanolone	312	248	288	378
Dehydroepiandrosterone	77	80	34	9
Epiandrosterone	4	48	14	12
11β-Hydroxyandrosterone	530	430	703	546
11β-Hydroxyaetiocholanolone	341	126	174	138

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	50
Name	David A.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	323308
Chronological age	17.1 years
Bone age	17.2 years
Height percentile	3
Pubertal stage	P3 G4
Clinical diagnosis	Short stature, hGH normal, delayed puberty
	After 1 month HCG/HMG treatment

URINARY CREATININE: 632-2,063 (mean 1,020) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.44 mg per 24 hours
Day 1	0.85
Day 2	1.26
Day 3	1.03

URINARY TESTOSTERONE

Basal	3.03 µg per 24 hours
Day 1	6.72
Day 2	13.98
Day 3	13.86

PLASMA TESTOSTERONE

Basal	47 ng per 100 ml
Day 1	100
Day 2	134
Day 3	202

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	3	14	9	10
5β-Androstane-3α,17β-diol	80	50	61	67
Δ^5-Androstene-3β,17β-diol	1	1	1	2
5α-Androstane-3β,17β-diol	1	1	1	1
Androsterone	250	230	504	623
Aetiocholanolone	151	181	395	578
Dehydroepiandrosterone	13	23	59	77
Epiandrosterone	5	4	7	6

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3. Basal day was 2 weeks after treatment.

Protocol	51
Name	Gordon B.
Hospital of admission	RHSC, Glasgow
Hospital number	176010
Chronological age	3.8 years
Bone age	3.8 years
Height percentile	50
Pubertal stage	P1 G1
Clinical diagnosis	Hypogonadism

URINARY CREATININE: 276-370 (mean 320) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.37 mg per 24 hours
Day 1	0.34
Day 2	0.40
Day 3	0.49

URINARY TESTOSTERONE

Basal	0.81 µg per 24 hours
Day 1	0.94
Day 2	1.78
Day 3	3.49

PLASMA TESTOSTERONE

Basal	157 ng per 100 ml
Day 3	924

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	13	12	7	5
5β-Androstane-3α,17β-diol	78	72	44	45
Δ^5-Androstene-3β,17β-diol	4	4	11	5
5α-Androstane-3β,17β-diol	5	3	2	2
Androsterone	56	55	72	107
Aetiocholanolone	40	118	59	86
Dehydroepiandrosterone	170	32	10	5
Epiandrosterone	81	19	9	4

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	52
Name	Hugh M.
Hospital of admission	RHSC, Glasgow
Hospital number	182897
Chronological age	6.8 years
Bone age	6.8 years
Height percentile	3
Pubertal stage	P1 G1
Clinical diagnosis	Short stature, hGH normal

URINARY CREATININE: 76-176 (mean 80) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.19 mg per 24 hours
Day 1	0.15
Day 2	0.20
Day 3	0.25

URINARY TESTOSTERONE

Basal	1.47 µg per 24 hours
Day 1	1.85
Day 2	1.99
Day 3	2.95

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	4	3	2	5
5β-Androstane-3α,17β-diol	51	33	27	163
Δ^5-Androstene-3β,17β-diol	3	3	2	8
5α-Androstane-3β,17β-diol	3	2	2	1
Androsterone	96	217	105	35
Aetiocholanolone	81	101	61	38
Dehydroepiandrosterone	7	11	3	108
Epiandrosterone	64	57	35	57

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	53
Name	Alan R.
Hospital of admission	RHSC, Glasgow
Hospital number	35487
Chronological age	10.9 years
Bone age	10.9 years
Height percentile	75
Pubertal stage	P1 G1
Clinical diagnosis	Bilateral undescended testes, gynaecomastia

URINARY CREATININE: 633-673 (mean 648) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.07 mg per 24 hours
Day 1	0.96
Day 2	0.72
Day 3	1.27

URINARY TESTOSTERONE

Basal	6.37 µg per 24 hours
Day 1	6.35
Day 2	9.76
Day 3	7.62

PLASMA TESTOSTERONE

Basal	857 ng per 100 ml
Day 3	2,870

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	29	25	29	30
5β-Androstane-3α,17β-diol	153	130	111	126
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	83	1,078	2,875	386
Androsterone	107	104	139	244
Aetiocholanolone	45	44	31	76
Dehydroepiandrosterone	ND	ND	ND	ND
Epiandrosterone	742	3,938	10,103	2,078

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	54
Name	Gordon W.
Hospital of admission	RHSC, Glasgow
Hospital number	160073
Chronological age	11.0 years
Bone age	12.7 years
Height percentile	90
Pubertal stage	P2 G2
Clinical diagnosis	Unilateral undescended testis

URINARY CREATININE: 442-906 (mean 525) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.71 mg per 24 hours
Day 1	0.82
Day 2	0.95
Day 3	1.30

URINARY TESTOSTERONE

Basal	0.99 µg per 24 hours
Day 1	NM
Day 2	NM
Day 3	4.27

PLASMA TESTOSTERONE

Basal	427 ng per 100 ml
Day 3	551

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	14	66	NM	NM
5β-Androstane-3α,17β-diol	308	329	346	240
Δ^5-Androstene-3β,17β-diol	NM	NM	NM	NM
5α-Androstane-3β,17β-diol	33	45	17	9
Androsterone	755	624	508	1,005
Aetiocholanolone	33	57	42	93
Dehydroepiandrosterone	96	123	144	94
Epiandrosterone	105	101	592	611

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	55
Name	Neil M.
Hospital of admission	RHSC, Glasgow
Hospital number	123788
Chronological age	12.6 years
Bone age	12.5 years
Height percentile	50
Pubertal stage	P2 G2
Clinical diagnosis	Unilateral undescended testis

URINARY CREATININE: 613-981 (mean 683) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.32 mg per 24 hours
Day 1	1.40
Day 2	1.84
Day 3	2.00

URINARY TESTOSTERONE

Basal	5.52 µg per 24 hours
Day 1	4.93
Day 2	NM
Day 3	20.40

PLASMA TESTOSTERONE

Basal	356 ng per 100 ml
Day 3	1,106

URINARY ANDROGEN METABOLITES

	µg per 24 hours	
	Basal	Day 1
5α-Androstane-3α,17β-diol	18	NM
5β-Androstane-3α,17β-diol	643	5,731
Δ^5-Androstene-3β,17β-diol	145	51
5α-Androstane-3β,17β-diol	62	21
Androsterone	705	405
Aetiocholanolone	597	221
Dehydroepiandrosterone	386	64
Epiandrosterone	53	7
11β-Hydroxyandrosterone	364	300
11β-Hydroxyaetiocholanolone	47	90

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	56
Name	Peter M.
Hospital of admission	RHSC, Glasgow
Hospital number	176803
Chronological age	13.7 years
Bone age	13.9 years
Height percentile	50
Pubertal stage	P2 G1
Clinical diagnosis	Obesity, hypogonadism

URINARY CREATININE		627-707 (mean 672) mg per 24 hours
URINARY 17-OXOSTEROIDS	Basal	0.79 mg per 24 hours
	Day 1	1.17
	Day 2	1.22
	Day 3	1.14
PLASMA TESTOSTERONE	Basal	109 ng per 100 ml
	Day 3	142

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	6	58	49	118
5β-Androstane-3α,17β-diol	86	237	187	264
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	3	3	19	17
Androsterone	609	1,008	961	1,433
Aetiocholanolone	379	500	605	981
Dehydroepiandrosterone	499	997	686	823
Epiandrosterone	247	532	334	533
11β-Hydroxyandrosterone	710	NM	NM	NM
11β-Hydroxyaetiocholanolone	18	NM	NM	NM

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	57
Name	John C.
Hospital of admission	RHSC, Glasgow
Hospital number	166792
Chronological age	13.9 years
Bone age	12.0 years
Height percentile	<3
Pubertal stage	P1 G2
Clinical diagnosis	Short stature, hGH normal, hypogonadism

URINARY CREATININE: 257-493 (mean 472) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.91 mg per 24 hours
Day 1	1.78
Day 2	1.16
Day 3	0.93

URINARY TESTOSTERONE

Basal	0.95 µg per 24 hours
Day 1	NM
Day 2	2.22
Day 3	2.68

PLASMA TESTOSTERONE

Basal	380 ng per 100 ml
Day 3	2,286

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	6	6	10	3
5β-Androstane-3α,17β-diol	65	114	73	29
Δ^5-Androstene-3β,17β-diol	23	18	17	10
5α-Androstane-3β,17β-diol	ND	ND	ND	ND
Androsterone	162	198	212	229
Aetiocholanolone	53	72	98	98
Dehydroepiandrosterone	98	112	136	225
Epiandrosterone	261	295	294	316

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	58
Name	Alasdair C.
Hospital of admission	RHSC, Glasgow
Hospital number	106507
Chronological age	4.5 years
Bone age	6.5 years
Height percentile	25-50
Pubertal stage	P1 G0
Clinical diagnosis	Prader-Willi syndrome, bilateral undescended testes

URINARY CREATININE: 432-508 (mean 480) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.56 mg per 24 hours
Day 1	0.70
Day 2	0.37
Day 3	0.62

URINARY TESTOSTERONE

Basal	1.03 µg per 24 hours
Day 1	1.09
Day 2	1.05
Day 3	1.55

PLASMA TESTOSTERONE

Basal	817 ng per 100 ml
Day 3	857

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	4	4	4	4
5β-Androstane-3α,17β-diol	ND	ND	ND	ND
Δ^5-Androstene-3β,17β-diol	24	54	34	48
5α-Androstane-3β,17β-diol	ND	ND	ND	ND
Androsterone	17	38	40	36
Aetiocholanolone	20	16	20	21
Dehydroepiandrosterone	ND	ND	ND	ND
Epiandrosterone	572	299	292	415

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	59
Name	Arshad R.
Hospital of admission	Luton & Dunstable Children's Annexe
Chronological age	5.4 years
Bone age	6.0 years
Height percentile	10-25
Pubertal stage	P1 G0
Clinical diagnosis	Laurence-Moon-Biedl syndrome, bilateral undescended testes

URINARY CREATININE: 247-304 (mean 272) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.15 mg per 24 hours
Day 1	0.70
Day 2	0.95
Day 3	0.73

URINARY TESTOSTERONE

Basal	0.29 µg per 24 hours
Day 1	0.32
Day 2	0.37
Day 3	0.46

PLASMA TESTOSTERONE

Basal	155 ng per 100 ml
Day 3	512

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	3	3	3	3
5β-Androstane-3α,17β-diol	28	70	22	30
Δ^5-Androstene-3β,17β-diol	22	66	26	23
5α-Androstane-3β,17β-diol	20	28	23	34
Androsterone	3	3	6	9
Aetiocholanolone	15	16	7	28
Dehydroepiandrosterone	3	12	4	11
Epiandrosterone	33	41	29	46
11β-Hydroxyandrosterone	198	203	192	209
11β-Hydroxyaetiocholanolone	89	94	64	39

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	60
Name	Scott M.
Hospital of admission	RHSC, Glasgow
Hospital number	183093
Chronological age	9.5 years
Bone age	9.5 years
Height percentile	50
Pubertal stage	P1 G0
Clinical diagnosis	Bilateral undescended testes after orchidopexy twice

URINARY CREATININE: 105-521 (mean 498) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.30 mg per 24 hours
Day 1	0.23
Day 2	0.38
Day 3	0.44

URINARY TESTOSTERONE

Basal	2.53 µg per 24 hours
Day 1	2.59
Day 2	2.65
Day 3	2.74

PLASMA TESTOSTERONE

Basal	50 ng per 100 ml
Day 3	253

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	5	9	3	4
5β-Androstane-3α,17β-diol	54	70	25	12
Δ^5-Androstene-3β,17β-diol	4	64	86	128
5α-Androstane-3β,17β-diol	16	23	85	181
Androsterone	15	83	104	67
Aetiocholanolone	8	44	20	20
Dehydroepiandrosterone	4	20	21	222
Epiandrosterone	57	80	55	6
11β-Hydroxyandrosterone	18	162	115	103
11β-Hydroxyaetiocholanolone	5	84	63	88

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	61
Name	Brian D.
Hospital of admission	RHSC, Glasgow
Hospital number	156169
Chronological age	9.6 years
Bone age	11.0 years
Height percentile	> 97
Pubertal stage	P1 G1
Clinical diagnosis	Unilateral undescended testis after surgery

URINARY CREATININE: 318-573 (mean 433) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.53 mg per 24 hours
Day 1	0.45
Day 2	0.22
Day 3	0.25

URINARY TESTOSTERONE

Basal	1.38 µg per 24 hours
Day 1	-
Day 2	1.62
Day 3	3.49

PLASMA TESTOSTERONE

Basal	177 ng per 100 ml
Day 3	1,098

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	6	6	2	12
5β-Androstane-3α,17β-diol	13	23	8	5
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	6	39	18	7
Androsterone	56	54	78	99
Aetiocholanolone	35	38	37	74
Dehydroepiandrosterone	72	17	20	137
Epiandrosterone	136	143	136	161

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	62
Name	Douglas S.
Hospital of admission	Southern General Hospital, Glasgow
Hospital number	156051
Chronological age	10.0 years
Height percentile	< 3
Pubertal stage	P1 G1
Clinical diagnosis	Short stature, hGH normal, bilateral undescended testes

URINARY CREATININE: 219-223 (mean 221) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.00 mg per 24 hours
Day 1	0.79
Day 2	0.66

URINARY TESTOSTERONE

Basal	0.56 µg per 24 hours
Day 1	1.88
Day 2	3.08

PLASMA TESTOSTERONE

Basal	398 ng per 100 ml
Day 2	768

URINARY ANDROGEN METABOLITES

	µg per 24 hours		
	Basal	Day 1	Day 2
5α-Androstane-3α,17β-diol	7	26	15
5β-Androstane-3α,17β-diol	21	16	28
Δ^5-Androstene-3β,17β-diol	ND	3	3
5α-Androstane-3β,17β-diol	9	19	8
Androsterone	23	21	30
Aetiocholanolone	10	8	14
Dehydroepiandrosterone	ND	ND	ND
Epiandrosterone	194	308	241
11β-Hydroxyandrosterone	39	NM	36
11β-Hydroxyaetiocholanolone	67	NM	78

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1 and 2.

Protocol	63
Name	William L.
Hospital of admission	RHSC, Glasgow
Hospital number	44279
Chronological age	10.9 years
Bone age	8.0 years
Height percentile	50
Pubertal stage	P1 G0
Clinical diagnosis	Bilateral undescended testes

URINARY CREATININE		469-621 (mean 541) mg per 24 hours
URINARY 17-OXOSTEROIDS	Basal	0.84 mg per 24 hours
	Day 1	0.73
	Day 2	0.67
	Day 3	0.50
URINARY TESTOSTERONE	Basal	0.64 µg per 24 hours
	Day 1	0.58
	Day 2	2.32
	Day 3	2.75
PLASMA TESTOSTERONE	Basal	336 ng per 100 ml
	Day 3	992

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	10	12	14	18
5β-Androstane-3α,17β-diol	4	6	11	15
Δ^5-Androstene-3β,17β-diol	4	4	5	7
5α-Androstane-3β,17β-diol	2	2	2	2
Androsterone	100	106	117	137
Aetiocholanolone	60	66	90	129
Dehydroepiandrosterone	229	158	201	242
Epiandrosterone	486	376	465	497

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	64
Name	Grant McF.
Hospital of admission	RHSC, Glasgow
Hospital number	164487
Chronological age	14.4 years
Bone age	12.1 years
Height percentile	<3
Pubertal stage	P1 G1
Clinical diagnosis	Short stature, hGH normal, delayed puberty

URINARY CREATININE: 354-606 (mean 452) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.71 mg per 24 hours
Day 1	0.35
Day 2	0.92
Day 3	0.45

URINARY TESTOSTERONE

Basal	0.72 µg per 24 hours
Day 1	1.08
Day 2	1.48
Day 3	1.94

PLASMA TESTOSTERONE

Basal	1,736 ng per 100 ml
Day 3	3,270

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	5	9	5	4
5β-Androstane-3α,17β-diol	37	39	41	17
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	12	43	47	21
Androsterone	157	180	311	326
Aetiocholanolone	60	64	165	164
Dehydroepiandrosterone	35	15	120	113
Epiandrosterone	214	240	154	158

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	65
Name	Edward M.
Hospital of admission	Gartnavel General Hospital, Glasgow
Hospital number	480603
Chronological age	15.0 years
Bone age	15.0 years
Height percentile	50
Pubertal stage	P3 G3
Clinical diagnosis	Cushing's syndrome

URINARY CREATININE: 741–894 (mean 782) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.91 mg per 24 hours
Day 1	0.41
Day 2	1.25
Day 3	5.19

URINARY TESTOSTERONE

Basal	5.47 µg per 24 hours
Day 1	5.52
Day 2	6.41
Day 3	7.49

PLASMA TESTOSTERONE

Basal	376 ng per 100 ml
Day 3	860

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	239	147	6	30
5β-Androstane-3α,17β-diol	243	215	134	155
Δ^5-Androstene-3β,17β-diol	19	17	21	10
5α-Androstane-3β,17β-diol	1	1	ND	1
Androsterone	505	632	767	732
Aetiocholanolone	715	992	1,478	1,182
Dehydroepiandrosterone	128	141	289	298
Epiandrosterone	36	40	60	42
11β-Hydroxyandrosterone	1,749	1,747	1,750	1,768
11β-Hydroxyaetiocholanolone	1,047	962	1,599	993

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1–3.

Protocol	66
Name	Paul C.
Hospital of admission	RHSC, Glasgow
Hospital number	172449
Chronological age	15.6 years
Bone age	15.5 years
Height percentile	50
Pubertal stage	P3 G3
Clinical diagnosis	Normal, early puberty

URINARY CREATININE: 263-778 (mean 611) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.99 mg per 24 hours
Day 1	3.17
Day 2	1.17
Day 3	1.80

URINARY TESTOSTERONE

Basal	9.92 µg per 24 hours
Day 1	10.64
Day 2	18.62
Day 3	19.58

PLASMA TESTOSTERONE

Basal	483 ng per 100 ml
Day 3	1,345

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	230	153	194	49
5β-Androstane-3α,17β-diol	75	59	67	58
Δ^5-Androstene-3β,17β-diol	23	2	21	76
5α-Androstane-3β,17β-diol	53	26	15	38
Androsterone	486	401	890	1,200
Aetiocholanolone	188	228	393	585
Dehydroepiandrosterone	92	79	123	90
Epiandrosterone	24	16	25	13

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	67
Name	David F.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	535688
Chronological age	20.0 years
Bone age	19.5 years
Height percentile	50
Pubertal stage	P5 G5
Clinical diagnosis	Normal

URINARY CREATININE: 569-734 (mean 654) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	5.69 mg per 24 hours
Day 1	5.40
Day 2	5.70
Day 3	6.50

URINARY TESTOSTERONE

Basal	14.26 µg per 24 hours
Day 1	18.81
Day 2	19.38
Day 3	15.76

PLASMA TESTOSTERONE

Basal	350 ng per 100 ml
Day 3	840

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	73	126	97	61
5β-Androstane-3α,17β-diol	168	133	115	116
Δ^5-Androstene-3β,17β-diol	216	148	157	114
5α-Androstane-3β,17β-diol	95	53	55	59
Androsterone	1,455	2,191	1,688	1,438
Aetiocholanolone	1,695	2,350	1,904	1,853
Dehydroepiandrosterone	79	327	311	226
Epiandrosterone	14	36	17	15

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	68
Name	Gordon A.
Hospital of admission	Outpatient, RHSC, Glasgow
Chronological age	26.0 years
Bone age	26.0 years
Height percentile	50
Pubertal stage	P5 G5
Clinical diagnosis	Normal male

URINARY CREATININE: 923–1,380 (mean 1,190) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	2.48 mg per 24 hours
Day 1	1.82
Day 2	3.94
Day 3	4.16

URINARY TESTOSTERONE

Basal	42.85 µg per 24 hours
Day 1	44.82
Day 2	61.90
Day 3	85.52

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	119	23	36	29
5β-Androstane-3α,17β-diol	114	80	215	225
Δ^5-Androstene-3β,17β-diol	3	2	5	3
5α-Androstane-3β,17β-diol	19	22	9	15
Androsterone	2,326	2,444	3,254	3,521
Aetiocholanolone	1,470	1,421	2,100	2,000
Dehydroepiandrosterone	446	255	531	501
Epiandrosterone	24	8	31	23
11β-Hydroxyandrosterone	1,100	1,105	1,090	1,108
11β-Hydroxyaetiocholanolone	177	110	138	110

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1–3.

Protocol	69
Name	David S.
Hospital of admission	RHSC, Glasgow
Hospital number	113005
Chronological age	12.1 years
Bone age	9.1 years
Height percentile	10
Pubertal stage	P1 G1
Clinical diagnosis	Bilateral undescended testes

URINARY CREATININE: 295–655 (mean 458) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.05 mg per 24 hours
Day 1	2.22
Day 2	1.21
Day 3	1.21

URINARY TESTOSTERONE

Basal	1.33 µg per 24 hours
Day 1	1.89
Day 2	4.00
Day 3	4.42

PLASMA TESTOSTERONE

Basal	1,238 ng per 100 ml
Day 3	11,305

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	223	61	NM	32
5β-Androstane-3α,17β-diol	144	275	NM	192
Δ^5-Androstene-3β,17β-diol) 5α-Androstane-3β,17β-diol))	9,538	1,176	81,432	500
Androsterone	444	262	275	250
Aetiocholanolone	169	193	287	363
Dehydroepiandrosterone	NM	NM	NM	NM
Epiandrosterone	353	NM	740	1,388
11β-Hydroxyandrosterone	2,282	2,197	2,341	2,242
11β-Hydroxyaetiocholanolone	242	124	189	344

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1–3.

Protocol	70
Name	Frederick S.
Hospital of admission	Gartnavel General Hospital, Glasgow
Hospital number	494378
Chronological age	19.9 years
Bone age	18.0 years
Height percentile	25-50
Pubertal stage	P2 G2
Clinical diagnosis	Cushing's syndrome, delayed puberty

URINARY CREATININE: 570-680 (mean 605) mg per 24 hours

URINARY TESTOSTERONE

Basal	3.84 µg per 24 hours
Day 1	4.76
Day 2	6.07
Day 3	8.02

PLASMA TESTOSTERONE

Basal	120 ng per 100 ml
Day 3	335

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	263	368	405	638
5β-Androstane-3α,17β-diol	440	462	703	814
Δ^5-Androstene-3β,17β-diol	300	272	314	197
5α-Androstane-3β,17β-diol	NM	146	175	126
Androsterone	1,498	1,873	1,933	2,703
Aetiocholanolone	444	700	854	1,410
Dehydroepiandrosterone	189	207	111	408
Epiandrosterone	291	260	236	276
11β-Hydroxyandrosterone	3,359	3,310	3,042	3,027

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	71
Name	Paul F.
Hospital of admission	RHSC, Glasgow
Hospital number	121995
Chronological age	6.3 years
Bone age	3.1 years
Height percentile	< 3
Pubertal stage	P1 G0
Clinical diagnosis	Short stature, hGH normal, bilateral undescended testes

URINARY CREATININE: 90-175 (mean 125) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.50 mg per 24 hours
Day 1	1.52
Day 2	0.49
Day 3	0.98

URINARY TESTOSTERONE

Basal	0.81 µg per 24 hours
Day 1	1.11
Day 2	1.41
Day 3	2.96

PLASMA TESTOSTERONE

Basal	86 ng per 100 ml
Day 3	318

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	50	73	60	73
5β-Androstane-3α,17β-diol	39	35	27	37
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	3	1	1	1
Androsterone	34	46	30	38
Aetiocholanolone	7	10	4	7
Dehydroepiandrosterone	10	10	3	9
Epiandrosterone	83	116	2	5

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	72
Name	Brian R.
Hospital of admission	Hawkhead Hospital, Paisley
Hospital number	147394
Chronological age	8.0 years
Bone age	6.5 years
Height percentile	10
Pubertal stage	P1 G1
Clinical diagnosis	? Prader-Willi syndrome

URINARY CREATININE	310-496 (mean 394) mg per 24 hours	
URINARY 17-OXOSTEROIDS	Basal	0.33 mg per 24 hours
	Day 1	0.13
	Day 2	0.21
	Day 3	0.10
URINARY TESTOSTERONE	Basal	2.25 µg per 24 hours
	Day 1	0.97
	Day 2	2.12
	Day 3	1.69
PLASMA TESTOSTERONE	Basal	317 ng per 100 ml
	Day 3	302

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	189	29	7	36
5β-Androstane-3α,17β-diol	23	9	6	10
Δ^5-Androstene-3β,17β-diol	ND	23	11	3
5α-Androstane-3β,17β-diol	42	38	202	39
Androsterone	194	49	104	103
Aetiocholanolone	7	18	15	19
Dehydroepiandrosterone	31	20	32	27
Epiandrosterone	6	5	16	11

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	73
Name	Melville M.
Hospital of admission	RHSC, Glasgow
Hospital number	175689
Chronological age	15.5 years
Bone age	12.6 years stationary
Height percentile	< 3
Pubertal stage	P2 G2
Clinical diagnosis	Short stature, hGH normal

URINARY CREATININE: 526-646 (mean 568) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.65 mg per 24 hours
Day 1	1.04
Day 2	1.58
Day 3	1.73

URINARY TESTOSTERONE

Basal	1.84 µg per 24 hours
Day 1	2.07
Day 2	2.79
Day 3	3.84

PLASMA TESTOSTERONE

Basal	139 ng per 100 ml
Day 3	520

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	149	NM	NM	NM
5β-Androstane-3α,17β-diol	9	NM	NM	NM
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	33	36	70	21
Androsterone	426	415	666	693
Aetiocholanolone	355	446	678	676
Dehydroepiandrosterone	87	68	98	76
Epiandrosterone	40	34	46	17
11β-Hydroxyandrosterone	403	388	606	442
11β-Hydroxyaetiocholanolone	438	410	280	399

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	74
Name	Ian S.
Hospital of admission	RHSC, Glasgow
Hospital number	162001
Chronological age	8.3 years
Height percentile	<3
Pubertal stage	P1 G1
Clinical diagnosis	Short stature, hGH normal

1

URINARY CREATININE: 174-313 (mean 246) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.50 mg per 24 hours
Day 1	0.90
Day 2	0.68
Day 3	0.18

URINARY TESTOSTERONE

Basal	3.25 µg per 24 hours
Day 2	2.41
Day 3	2.85

URINARY ANDROGEN METABOLITES

µg per 24 hours

	Basal	Day 2	Day 3
5α-Androstane-3α,17β-diol	2	2	4
5β-Androstane-3α,17β-diol	154	88	144
Δ^5-Androstene-3β,17β-diol	11	4	3
5α-Androstane-3β,17β-diol	277	18	67
Androsterone	70	72	106
Aetiocholanolone	27	36	49
Dehydroepiandrosterone	7	3	6
Epiandrosterone	1	6	2

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	75
Name	James S.
Hospital of admission	RHSC, Glasgow
Hospital number	142333
Chronological age	7.0 years
Bone age	7.0 years
Height percentile	25-50
Pubertal stage	P1 G1
Clinical diagnosis	Bilateral undescended testes

URINARY CREATININE: 310-458 (mean 426) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.79 mg per 24 hours
Day 1	0.66
Day 2	0.84
Day 3	0.64

URINARY TESTOSTERONE

Basal	3.14 µg per 24 hours
Day 1	2.67
Day 2	6.86
Day 3	7.43

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	34	9	3	22
5β-Androstane-3α,17β-diol	116	98	53	50
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	2	1	2	1
Androsterone	60	49	46	41
Aetiocholanolone	10	16	37	19
Dehydroepiandrosterone	ND	ND	ND	ND
Epiandrosterone	23	21	12	17
11β-Hydroxyandrosterone	266	335	406	274
11β-Hydroxyaetiocholanolone	259	280	266	188

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	76
Name	Iain S.
Hospital of admission	RHSC, Glasgow
Hospital number	178150
Chronological age	9.0 years
Bone age	9.0 years
Height percentile	50
Pubertal stage	P1 G1
Clinical diagnosis	Hypogonadism

URINARY CREATININE: 500-745 (mean 625) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.87 mg per 24 hours
Day 1	0.65
Day 2	0.90
Day 3	1.06

URINARY TESTOSTERONE

Basal	4.26 µg per 24 hours
Day 1	3.37
Day 2	2.46
Day 3	2.05

PLASMA TESTOSTERONE

Basal	106 ng per 100 ml
Day 3	248

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	21	4	13	11
5β-Androstane-3α,17β-diol	66	50	89	106
Δ^5-Androstene-3β,17β-diol	4	3	4	4
5α-Androstane-3β,17β-diol	65	116	151	208
Androsterone	316	271	384	426
Aetiocholanolone	79	73	101	116
Dehydroepiandrosterone	28	38	9	11
Epiandrosterone	119	13	17	20

Patient was administered HCG (6,000 I.U. per day) intramuscularly on days 1-3.

Protocol	77
Name	Jeremy W.
Hospital of admission	Royal Manchester Children's Hospital, Manchester
Hospital number	74760
Chronological age	9.5 years
Bone age	14.0 years
Height percentile	>97
Pubertal stage	P4 G4
Clinical diagnosis	Precocious puberty (pineal tumour)

URINARY CREATININE: 719-915 (mean 820) mg per 24 hours

URINARY TESTOSTERONE

Basal	9.8 µg per 24 hours
Day 1	1.82
Day 2	1.42
Day 3	0.61
Day 4	0.96
Day 5	0.43

PLASMA TESTOSTERONE

Basal	28,900 ng per 100 ml
Day 1	394
Day 3	992
Day 4	716

URINARY ANDROGEN METABOLITES — µg per 24 hours

	Day 1	Day 2	Day 3	Day 4	Day 5
5α-Androstane-3α,17β-diol	19	13	25	13	6
5β-Androstane-3α,17β-diol	27	80	110	56	29
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	1	64	220	179	49
Androsterone	1,439	50	35	145	11
Aetiocholanolone	128	10	45	51	64
Dehydroepiandrosterone	364	53	41	148	55
Epiandrosterone	425	127	44	96	48

Basal day (22.11.73) pretreatment and diagnosis of tumour.
Day 1 (10.1.74) treatment with cyproterone acetate.
Day 2 (22.1.74) tumour removed.
Day 3 (30.1.74) LHRH (100 µg I.V.).
Day 4 (6.2.74) general patient review.
Day 5 (19.3.74) general patient review.

Protocol	78
Name	David D.
Hospital of admission	RHSC, Glasgow
Hospital number	173987
Chronological age	5.0 years
Bone age	10.5 years
Height percentile	>97
Pubertal stage	P3 G3
Clinical diagnosis	Precocious puberty, testicular tumour?

URINARY CREATININE: 458-600 (mean 507) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	0.20 mg per 24 hours
Day 1	0.39
Day 2	0.11
Day 3	0.39
Day 4	0.26

URINARY TESTOSTERONE: Basal 22.75 µg per 24 hours

PLASMA TESTOSTERONE: Basal 4,452 ng per 100 ml

PLASMA DEHYDROEPIANDROSTERONE: Basal 21 µg per 100 ml

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Basal	Day 1	Day 2	Day 3	Day 4
5α-Androstane-3α,17β-diol	2	4	19	172	53
5β-Androstane-3α,17β-diol	805	84	213	193	364
Δ^5-Androstene-3β,17β-diol	ND	ND	ND	ND	ND
5α-Androstane-3β,17β-diol	2	6	3	8	4
Androsterone	325	317	202	431	372
Aetiocholanolone	58	51	32	105	141
Dehydroepiandrosterone	5	1	1	ND	11
Epiandrosterone	22	7	6	17	22
11β-Hydroxyandrosterone	324	489	103	493	660
11β-Hydroxyaetiocholanolone	40	60	17	ND	ND

Patient was administered Dexamethazone (0.5 mg, 4 times daily, orally on days 1 and 2; 2.0 mg, 4 times daily, orally on days 3 and 4).

Protocol	79
Name	David A.
Hospital of admission	Western Infirmary, Glasgow
Hospital number	323308
Chronological age	17.2 years
Bone age	17.2 years
Height percentile	3
Pubertal stage	P3 G4
Clinical diagnosis	Short stature, hGH normal, delayed puberty Last week of HCG/HMG treatment

URINARY CREATININE: 632-2,063 (mean 1,020) mg per 24 hours

URINARY 17-OXOSTEROIDS

Day 1	1.67 mg per 24 hours
Day 2	1.31
Day 3	0.95
Day 4	1.99

URINARY TESTOSTERONE

Day 1	6.60 µg per 24 hours
Day 2	3.23
Day 3	8.19
Day 4	5.85

PLASMA TESTOSTERONE

Day 1	40 ng per 100 ml
Day 3	89

URINARY ANDROGEN METABOLITES: µg per 24 hours

	Day 1	Day 2	Day 3	Day 4
5α-Androstane-3α,17β-diol	14	14	14	9
5β-Androstane-3α,17β-diol	70	110	91	89
Δ^5-Androstene-3β,17β-diol	1	2	1	2
5α-Androstane-3β,17β-diol	1	2	1	2
Androsterone	281	221	259	219
Aetiocholanolone	242	201	226	206
Dehydroepiandrosterone	40	37	50	36
Epiandrosterone	10	18	12	6

Patient was administered HCG (1,600 I.U. per day) intramuscularly on days 1 and 3 and HMG (1,000 I.U. per day) on days 2 and 4.

Protocol	80
Name	Alexander G.
Hospital of admission	RHSC, Glasgow
Hospital number	16663
Chronological age	14.0 years
Bone age	14.0 years
Height percentile	10-25
Pubertal stage	P2 G3
Clinical diagnosis	Gynaecomastia, XX-male
Karyotype	46,XX

URINARY CREATININE: 1,034-1,392 (mean 1,191) mg per 24 hours

URINARY 17-OXOSTEROIDS

Basal	1.46 mg per 24 hours
Day 1	1.63
Day 2	2.92
Day 3	3.71

URINARY ANDROGEN METABOLITES

	µg per 24 hours			
	Basal	Day 1	Day 2	Day 3
5α-Androstane-3α,17β-diol	ND	ND	ND	ND
5β-Androstane-3α,17β-diol	18	9	19	20
Δ^5-Androstene-3β,17β-diol	14	14	26	58
5α-Androstane-3β,17β-diol	ND	ND	ND	ND
Androsterone	933	1,113	735	745
Aetiocholanolone	421	439	378	417
Dehydroepiandrosterone	502	403	635	792
Epiandrosterone	34	31	66	89

Patient was administered Corticotrophin (ACTH; 40 mg per day) intramuscularly on days 1-3.

CHAPTER 6

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THE URINARY METABOLITES OF TESTOSTERONE: AN INDEX OF TESTICULAR FUNCTION IN CHILDREN

by

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A SUMMARY

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The evaluation of testicular Leydig cell function is of considerable clinical value in the investigation of gonadal problems in childhood and adolescence. Testicular function was assessed in the patients described in this thesis by the estimation of the basal plasma testosterone concentration, the basal daily urinary excretion of testosterone and the individual androgen metabolites and the changes in these parameters to administered HCG. This method therefore not only gives an index of testosterone production but also allows investigation of actual testosterone utilization by the patient.

Following the addition of [1,2,6,7-^{3}H]testosterone for recovery purposes, aliquots of plasma were made alkaline by the addition of sodium hydroxide and the testosterone extracted with diethyl ether. Purification of the extracted residues was effected by partition and thin layer chromatography. The dried residues were dissolved in dichloromethane and esterified with heptafluorobutyric anhydride for 30 minutes at room temperature. Following subsequent purification by thin layer chromatography, quantitation of testosterone-17-heptafluorobutyrate was made by electron capture detection on a Pye 104 gas liquid chromatograph. A correction was made for the recovery rate of radioactively labelled testosterone added initially to plasma samples.

A near total hydrolysis of urinary conjugates of testosterone was effected by incubation with β-glucuronidase (750 F.u. per ml urine) at 37^{o}C for 72 hours without inhibition of the sulphatase activity

of the enzyme preparation. Following the addition of [1,2,6,7-^{3}H]-testosterone and extraction with diethyl ether, the urinary residues were purified, esterified and quantitated as for the plasma extracts.

Similar hydrolysed and extracted urinary residues were purified by partition only. The dried residues were dissolved in dichloromethane and esterified with heptafluorobutyric anhydride at room temperature for 30 minutes. Quantitation of the individual androgen esters was made from a tracing of various androgens as obtained from flame ionization detection of the compounds in a Pye 104 gas liquid chromatograph.

All urine specimens were subjected to routine 17-oxosteroid determination by the Zimmermann reaction and creatinine determination by the Jaffé reaction. All urine results were subsequently adjusted to a constant daily creatinine excretion over the period of collection.

The results from 80 patients investigated (age range 5 months to 26 years) were analysed against chronological age, height and pubertal status of the patients. Patients with bilateral undescended testes had a greatly elevated urinary excretion of epiandrosterone, implying abnormal androgen metabolism, and a significantly subnormal or undetectable urinary excretion of dehydroepiandrosterone and Δ^{5}-androstenediol, implying a deficiency of 17,20-desmolase. Patients with hypogonadism also had a significantly subnormal or undetectable urinary excretion of Δ^{5}-androstenediol but in combination with a normal urinary excretion of dehydroepiandrosterone, implying a deficiency of 17β-reductase.

Patients with short stature, bilateral undescended testes, hypogonadism (microgenitalia) or delayed puberty had a significantly subnormal urinary excretion of testosterone in combination with a normal or elevated plasma testosterone concentration. Partial tissue insensitivity to testosterone and increased binding to testosterone binding globulin were advanced as possible explanations of the testosterone levels in these patients and these possibilities were discussed.

Testicular function was assessed in nine patients with abnormal sex chromosome constitution. Two patients with Klinefelter's syndrome (47,XXY and 46,XX) had poor production and utilization of testosterone. Four patients with the testicular feminization syndrome had good testosterone production and variable, although generally poor, utilization of testosterone, this suggesting partial or complete tissue insensitivity to testosterone, the accepted explanation of this syndrome. Two patients with the XO/XY mosaicism and female phenotype (unilateral testis and contralateral streak gonad) had 'near-normal' production of testosterone whilst a patient with the XX/XY genotype and bilateral ovotestes had extremely poor production and poor utilization of testosterone.

Clinical significance was given to the urinary excretion of three metabolites - 5α-androstanediol as an index of androgenicity, 5β-androstanediol as an index of masculine genital development and epiandrosterone as an index of the somatic utilization of testosterone.

The possibility that the urinary excretion of these metabolites may have prognostic significance in prepubertal patients was discussed.