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CHANGES IN THE PITUITARY–TESTICULAR SYSTEM WITH AGE

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SUMMARY

In order to provide a comprehensive account of pituitary–testicular function in man, 466 subjects, ranging in age from 2 to 101 years, were studied to examine blood levels of the pituitary gonadotrophins (LH and FSH), the sex steroids testosterone and oestradiol, the binding capacity of the sex hormone binding globulin (SHBG), the free testosterone and oestradiol fractions, and the transfer constant for the peripheral conversion of testosterone to oestradiol. The results were compared with clinical indices of testicular size, sexual function and secondary sex hair distribution. Serum LH and FSH were low before puberty, increased in pubertal adolescents to levels somewhat above those of adults and subsequently increased progressively over the age of 40 years. Testosterone levels fell slowly after the age of 40, while there was a slight rise in plasma oestradiol with increasing age. FSH and testosterone showed small seasonal variations in young adult men, the lowest values being seen in winter. SHBG binding capacity was high in two pre-pubertal boys, fell in adult men, but increased in old age. Free testosterone and oestradiol levels fell in old age. The metabolic clearance rates (MCR) of testosterone and oestradiol also fell in old age, while the conversion of testosterone to oestradiol was increased. Many correlations were observed between various hormonal and clinical measurements. The evidence is consistent with a primary decrease in testicular function over the age of 40 years.

INTRODUCTION

While it is common knowledge that testicular function declines in old age, relatively few detailed studies of the hormonal aspects of this senescent change have been made with modern techniques. Kinsey *et al.* (1948) found a progressive decrease in sexual activity with

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age and at the age of 80 years approximately 75% of men were impotent. Secondary sexual hair decreases with age (Hamilton, 1951; Melick & Taft, 1959) while reduced testicular size and histological features of impaired spermatogenesis have been found in old men (Bennett *et al.*, 1950).

It has been known for some time that in men the pituitary content of gonadotrophins increases with age (Henderson & Rowlands, 1938). There is a sharp rise in urinary gonadotrophin excretion in men over the age of 60 years and a more gradual progressive rise may commence in the fourth decade (Albert, 1956; Johnsen, 1959). The urinary excretion of 17-oxosteroids and testosterone glucuronide falls with advancing age and testosterone levels in spermatic vein blood have been found to be lower in old men than in young men (Engle & Pincus, 1956; Hollander & Hollander, 1958; Morer-Fargas & Nowakowski, 1965).

The present report describes an examination of changes which occur in the male reproductive system with advancing age and includes some data from children and adolescents. The blood levels of LH and FSH, testosterone and oestradiol, the binding capacity of SHBG, the free testosterone and oestradiol fractions, the MCR of testosterone and oestradiol and the transfer constants for the peripheral conversion of testosterone to oestradiol have been determined in males of different ages. The results have been compared with clinical indices of testicular size, sexual function and secondary sex hair distribution. Furthermore, the possibility of seasonal variations in the endocrine parameters was examined over the course of 1 year

METHODS

Subjects

The blood levels of LH, FSH, testosterone and oestradiol were measured in a total of 466 subjects and divided into several groups (Table 1). No attempt was made to collect the blood samples at any particular time of day.

The prepubertal children included patients in hospital convalescing from acute illness and boys presenting for treatment of short stature in whom investigations revealed no endocrine cause for growth retardation. Thus, in these subjects, the hormone levels were measured in blood samples collected for other purposes. The pubertal and adolescent schoolboy volunteers were participating in a study of pubertal development and blood lipid levels. The adult and other adolescent volunteers were students or members of staff.

Most of the men over the age of 70 years were ambulant patients from a geriatric hospital. They had been in hospital for periods of from 5 months to 13 years and had senescent disorders. All were in a fair state of general health at the time of study. Most were being given hypnotics and some were taking tranquillizers or other medications, but none was receiving hormone preparations.

The men with normal sperm counts were either volunteer semen donors for artificial insemination or apparently healthy partners of infertile marriages. The orthopaedic inpatients were men convalescing from uncomplicated fractures.

The possibility of annual variations in blood levels of LH, FSH, testosterone and oestradiol in men was examined in twenty-six subjects who donated blood at 3-monthly intervals for 1 year. The samples were classified and collected between the following dates: winter, 1–3 June; spring, 31 August to 3 September; summer, 24 November to 7 December; autumn, 22 February to 10 March. Two-way analyses of variance (subjects × seasons) were performed for each hormone.

TABLE 1. Subjects studied

Subjects	No.	Group	No.
Prepubertal boys	31	Hospital inpatients	17
		Idiopathic short stature	9
		Volunteers	5
Pubertal boys	58	Schoolboy volunteers	48
		Idiopathic short stature	10
Mature adolescents (14–21 years)	104	Schoolboy volunteers	85
		Blood donors	11
		Other volunteers	8
Adults (21–101 years)	273	Blood donors	121
		Volunteers	59
		Geriatric inpatients	51
		Men with known normal sperm counts	29
		Orthopaedic inpatients	13

Clinical evaluation

In the children, stages of genital and pubic hair development were assessed by the method of Tanner (1962). Testicular volume was measured with an orchidometer (Prader, 1966) and in the adults, pubic, axillary, beard, trunk and limb hair was examined and the patient asked whether there had been any reduction in hair growth in these regions. Secondary sex hair was recorded as normal, mildly, moderately or severely reduced or absent. Assessment of sexual function was difficult because of the wide range of sexual activity in men, the changes with age and particularly because of varying attitudes of different men to discussion of the topic. Subjects were questioned about the frequency of penile erections and seminal emissions, both spontaneous and during sexual arousal and whether they considered their sexual function to be normal or reduced. They were also asked about sex drive or urge. From the answers to these questions, sexual function was recorded as normal, reduced or absent. The blood donors were not examined.

Hormone assays

Serum LH and FSH concentrations were measured by specific double antibody radioimmunoassays (Alford *et al.*, 1973). A laboratory preparation of human pituitary gonadotrophin with bioassay potencies of 40 iu 2nd IRP HMG/mg FSH, and 144 iu/mg LH was used as a standard in both assays and the results are expressed in iu equivalents of 2nd IRP HMG/ml serum. In these assays 1 μg LER 907 was equivalent to 20 miu FSH and 40 miu LH. The precision of these assays, expressed as the coefficient of variation, was 6% within and 15% between assays. No other anterior pituitary hormones showed significant cross-reaction in these assays.

In the early phases of this study plasma testosterone was measured by a competitive protein method using SHBG from last trimester pregnancy plasma (Baker *et al.*, 1973); in the majority of samples testosterone was measured by radioimmunoassay (Wang *et al.*, 1974). The two methods gave results that were not significantly different. In both assays between 40 and 50% of circulating dihydrotestosterone was measured as testosterone, since

both SHBG and testosterone antibody bind dihydrotestosterone. Plasma oestradiol was measured by radioimmunoassay using an antibody raised against 6-keto oestradiol coupled with thyroglobulin. This antibody has a cross reactivity of less than 15% with other oestrogens from which oestradiol is separated by chromatography (Dufau *et al.*, 1970). The precision of both these assays expressed as a coefficient of variation was 10% within and 17% between assays.

Plasma protein binding of testosterone and oestradiol

The SHBG capacity and free testosterone and oestradiol fractions in diluted plasma at 4°C were estimated by an ammonium sulphate precipitation (ASP) method (O'Connor *et al.*, 1973) in sixty-two adults aged between 22 and 99 years, in two prepubertal boys aged 7 and 10 years and in four pools of plasma from pubertal subjects.

Metabolic clearance rates

MCR^{T} was determined in seventeen and MCR^{E_2} in fifteen men aged between 21 and 83 years. The clearance rates of both steroids were measured in eight and the transfer constant, $\rho_{pp}^{TE_2}$, estimated in seven. Eight of the subjects were orthopaedic inpatients.

MCR^{T} was measured using the constant infusion technique of Horton & Tait (1966) and MCR^{E_2} by the method of Longcope *et al.* (1968). Production rates of testosterone (P_p^{T}) and oestradiol ($P_p^{E_2}$) were derived from the products of the MCR and plasma concentrations of the steroid. Conversion ratios of testosterone to oestradiol ($C_{pp}^{TE_2}$) were measured by the method of Longcope *et al.* (1969) and the transfer constants ($\rho_{pp}^{TE_2}$) were calculated using the MCR^{E_2} data obtained in a separate study. Patients were fasting and recumbent for 12 h before the start of the infusion which commenced between 07.00 and 09.00 hours.

Statistical analysis

The form of the distributions of LH, FSH, testosterone and oestradiol levels was examined using the coefficient of skewness (Snedecor & Cochran, 1967). This method overcomes the problems of grouping results for the χ^2 test of goodness of fit and provides additional data. Student's t tests were used to examine the significance of differences between results of different age groups. The χ^2 statistic was used to test the differences in proportions. Correlation coefficients (r) were calculated for regressions between variables by the method of least squares. Because the distributions of LH, FSH and testosterone levels appeared to be best approximated by lognormal distributions in most groups tested, 95% confidence interval ranges have been calculated and statistical tests have been performed after logarithmic transformation. Where there were two or more results from the above measurements for individual subjects the mean has been used in the statistical analyses.

RESULTS

The data are presented in the above sequence but sections on the interrelationships of measurements in adults and adolescents have been dealt with separately. For this purpose, the age of 21 years has been taken as the dividing line between adolescence and adulthood. Further, because the age of the adult subjects affected the results, two age groups (21–50 years and 51–101 years) have been selected for comparison.

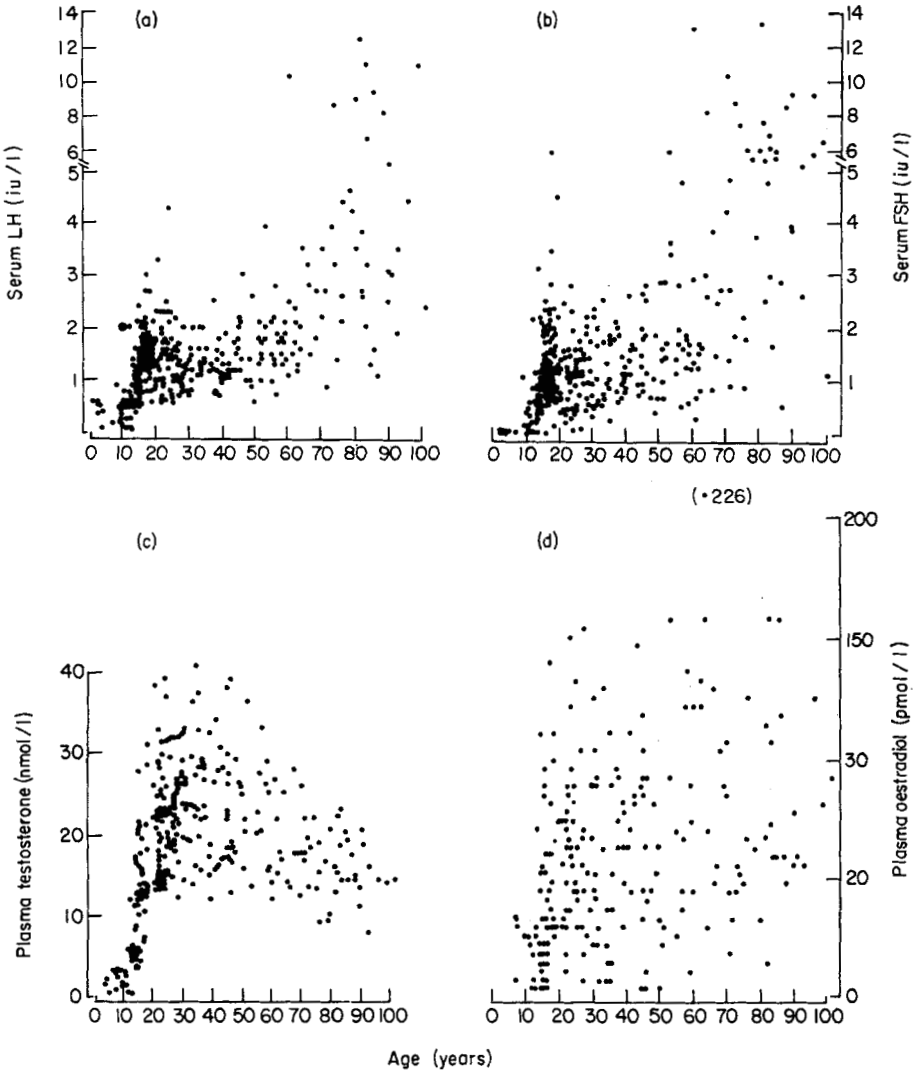


FIG. 1. (a) Serum LH (iu/l), (b) serum FSH (iu/l), (c) testosterone (nmol/l), and (d) oestradiol (pmol/l), as a function of age in normal males.

Results of single measurements in males of different ages—normal ranges

Serum LH and FSH (Figs. 1a and 1b). Changes in levels of LH and FSH presented similar patterns with increasing age of the subjects. Levels were low in prepubertal children and higher in pubertal adolescents aged between 11 and 17 years. Of the thirty-seven men over the age of 70 years, twenty-two had LH levels above the upper limit of the range for men aged between 21 and 50 years and fifteen had levels within the normal range. FSH levels were elevated in twenty-five men over the age of 70 years. The LH and FSH levels in men over the age of 50 years (mean LH 3·04, FSH 7·27 iu/l) were significantly higher than in men

TABLE 2. Seasonal study: mean LH, FSH, testosterone and oestradiol levels

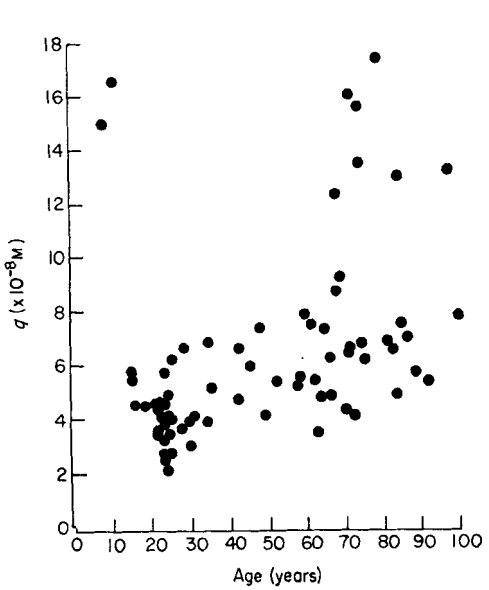
	Winter	Spring	Summer	Autumn	D^*
LH (iu/l)	1·48	1·66	1·65	1·77	0·29
FSH (iu/l)	2·09	2·35	2·22	2·28	0·24
Testosterone (nmol/l)	25·0	25·5	25·8	28·8	1·08
Oestradiol (pmol/l)	66·1	86·9	60·3	60·1	8·10

* $D = Q_{0.05} \times S\bar{x}$.

aged between 21 and 50 years (LH 1·42, FSH 1·95 iu/l) ($P<0·001$). The distribution of gonadotrophin levels was lognormal.

Plasma testosterone (Fig. 1c). There was a progressive rise in the concentrations of testosterone in plasma from subjects after the age of 10 years and this rise continued into the third decade. From the age of 40 years there was a steady decline with advancing age. While only five of the thirty-seven men over the age of 70 years had levels below the lowest seen in men aged between 21 and 50 years, none had levels above 24 nmol/l. The distribution of testosterone levels from adult men aged between 21 and 70 years was also best approximated by a lognormal distribution. Men over the age of 50 years had significantly lower levels of testosterone (mean 17·5 nmol/l) than did the younger men (mean 22·0 nmol/l; $P<0·001$).

Plasma oestradiol (Fig. 1d). Prepubertal children had low levels of oestradiol in plasma. After puberty, the results were widely scattered; there appeared to be a rise with advancing age but the difference between the levels from young and old men was relatively small. The mean levels in the two groups were 59·2 and 77·8 pmol/l ($P<0·005$).



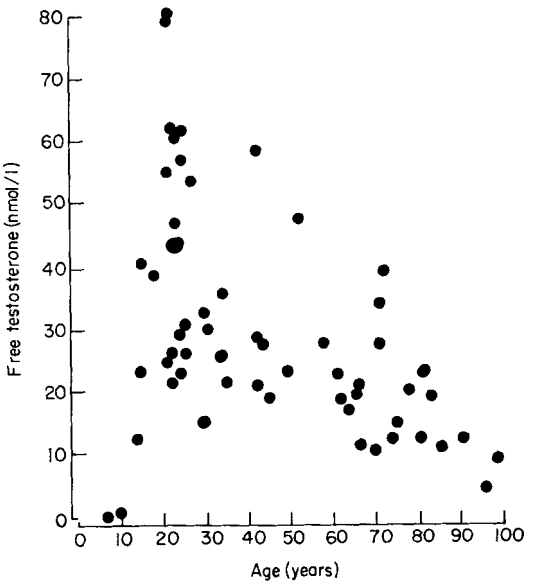


FIG. 2. SHBG binding capacity ($q \times 10^{-8}$ M) as a function of age in normal males.

FIG. 3. Plasma free testosterone concentration (nmol/l) as a function of age in normal males.

Seasonal effects of LH, FSH, testosterone and oestradiol. There were significant differences in the levels of each hormone between subjects, but the between seasons effect was only significant for FSH and testosterone levels ($P<0{\cdot}05$, Table 2). Both appeared to be lower in winter and when tested by the Q method (Snedecor & Cochran, 1967) there were significant differences between the mean FSH levels for winter and spring, and the mean testosterone levels for winter and autumn and also between the mean LH levels for winter and autumn (Table 2). The highest mean oestradiol level was in the spring but there were no significant differences between pairs of means.

SHBG binding capacity (Fig. 2). The SHBG binding capacity (q) was high in the two prepubertal boys and low in subjects who had commenced puberty. Levels gradually increased with the advancing age in adults and high levels were seen in eleven of nineteen men over

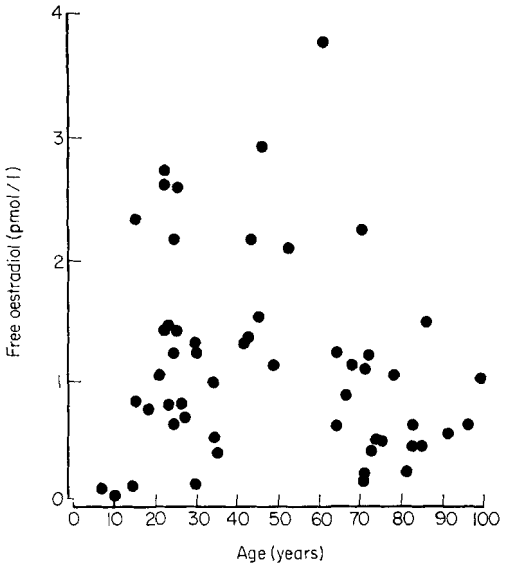


FIG. 4. Plasma free oestradiol concentration (pmol/l) as a function of age in normal males.

the age of 70 years. The mean levels from adults under and over the age of 50 years were $4{\cdot}3\times10^{-8}$ M and $7{\cdot}8\times10^{-8}$ M ($P<0{\cdot}001$).

The free steroid levels in plasma were calculated from the total levels and fractional free testosterone or oestradiol. The percentage free testosterone and oestradiol were significantly lower in adults after the age of 50 years (1·7 *v* 1·1% and 2·6 *v* 1·5% respectively; both $P<0{\cdot}005$). Free testosterone levels were low in prepubertal boys and in men over the age of 90 years (Fig. 3). Highest levels were seen in the 20–30 year age group and there was a downward trend with age in adults. Mean free testosterone was 0·38 nmol/l in men 21–50 years and 0·19 nmol/l in men 51–101 years of age. The oestradiol levels were more widely scattered, low levels being seen in prepubertal children, in men over the age of 70 years and in one subject aged 30 years (Fig. 4). Mean free oestradiol was 1·44 pmol/l in the younger age group and 0·95 pmol/l in older men ($P<0{\cdot}05$).

MCR^T and MCR^{E_2} (Fig. 5). The MCR results were calculated as l/24 h and l/24 h/m^2.

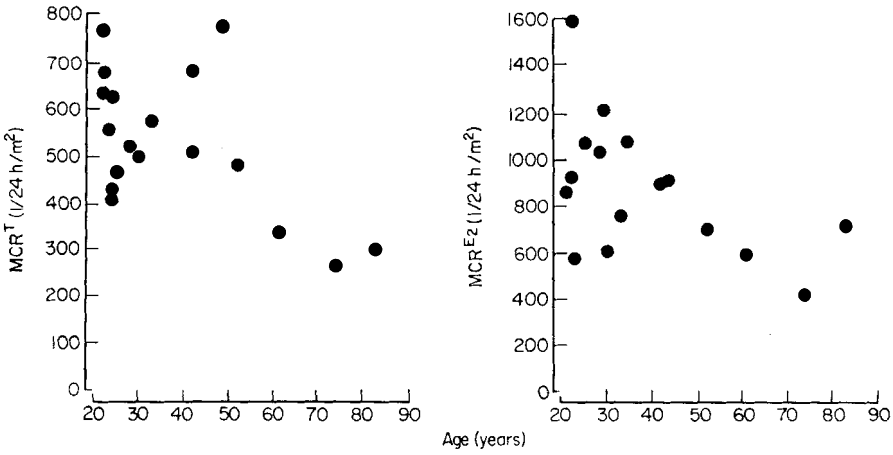


FIG. 5. Metabolic clearance rates for testosterone (MCR^T) and oestradiol (MCR^{E_2}), expressed in terms of body surface area (l/24 h/m^2) as a function of age in normal males.

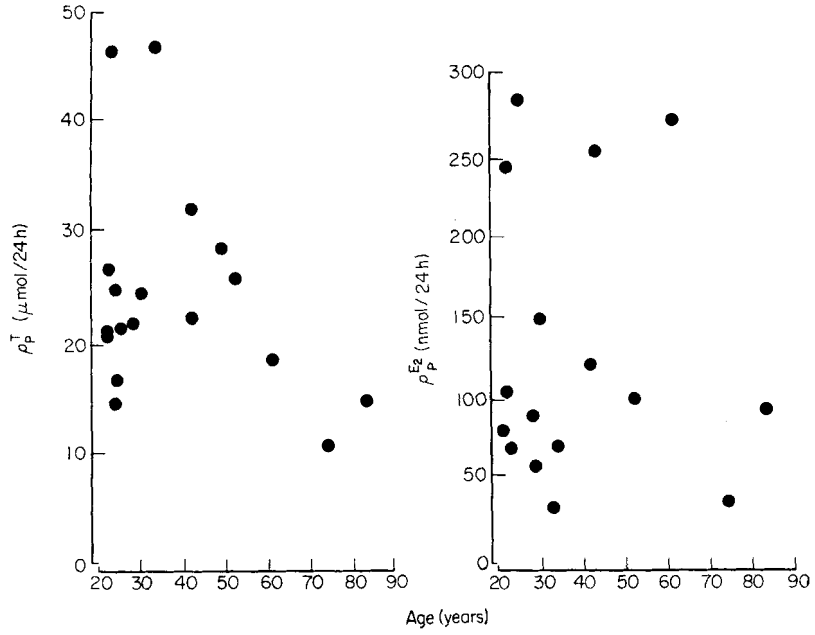


FIG. 6. Plasma production rate of testosterone (P^T, μmol/24 h) and oestradiol (P^{E_2} nmol/24 h) as a function of age in normal males.

Although only a small number of subjects was studied, values tended to be lower with advancing age and the mean MCR of both steroids in the four men over the age of 50 years were significantly lower than the mean results for the younger group (MCR^T: 587 *v* 363 l/24 h/m^2, $P<0{\cdot}005$; MCR^{E_2}: 938 *v* 613 l/24 h/m^2; $P<0{\cdot}05$).

P_p^T and $P_p^{E_2}$ (Fig. 6). In the younger men the P_p^T results were between 13·5 and 44·1 μmol/24 h. The values decreased with advancing age but the difference between the means for the

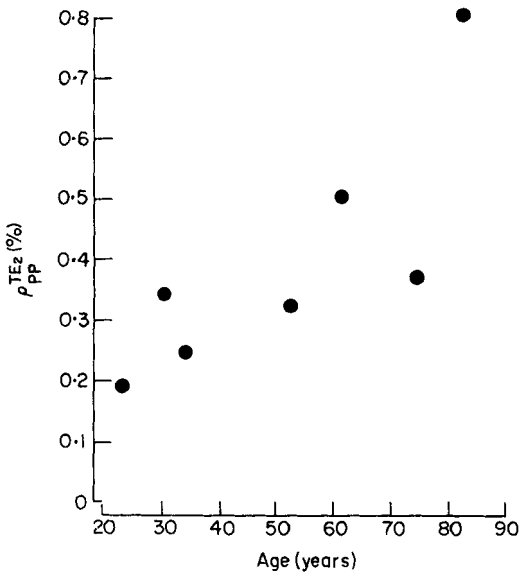


FIG. 7. Transfer constants for the peripheral conversion of testosterone to oestradiol ($\rho_{pp}^{TE}{}_2$, %) as a function of age in normal males.

men under and over the age of 50 years was only significant at the 10% level (25·0 *v* 16·3 μmol/24 h). $P_p^{E_2}$ results were widely scattered. They were not obviously affected by the age of the subjects and the difference in the two age groups was not significant (133 *v* 126 nmol/24 h).

$\rho_{pp}^{TE_2}$ (Fig. 7). The $\rho_{pp}^{TE_2}$ values rose with age and the oldest men studied (83 years) had a $\rho_{pp}^{TE_2}$ three times higher than the mean for the men aged between 21 and 50 years. Although the results for men under and over 50 years of age were not significantly different (0·27 *v*

TABLE 3. Clinical measurements in children and adolescents (mean ± SD)

	State of genital development				
	I	II	III	IV	V
No.	31	21	19	54	120
Age (years)	8·5 ±3·4	13·0 ±1·0	14·4 ±1·2	15·5 ±1·3	16·6 ±1·3
Stage of pubic hair development	I	1·2 ±0·4	2·5 ±0·9	4·4 ±0·8	5·2 ±0·7
Testicular volume (ml)	2·3 ±0·8	5·2 ±3·0	14·6 ±3·8	18·6 ±4·1	22·7 ±3·3
Height (cm)	—	152·2 ±5·3	162·3 ±7·5	170·1 ±7·4	174·3 ±9·1
Weight (kg)	—	41·6 ±6·8	50·1 ±6·6	57·9 ±7·6	64·9 ±7·9

TABLE 4. Mean (±SD) hormone levels in children and adolescents related to stage of genital development

	State of genital development				
	I	II	III	IV	V
LH (iu/l)	0·5±0·2 (21)	0·8±0·5 (16)	1·2±0·7 (13)	1·5±0·5 (21)	1·6±0·4 (102)
FSH (iu/l)	0·5±0·5 (21)	1·8±1·2 (15)	1·2±0·6 (13)	2·4±1·3 (21)	2·2±1·4 (102)
Testosterone (nmol/l)	1·9±1·0 (16)	6·5±2·5 (10)	7·8±3·5 (13)	13·6±2·7 (13)	16·9±6·3 (22)
Oestradiol (pmol/l)	19·3±11·5 (9)	19·3±17·8 (6)	24·8±18·9 (11)	33·0±30·3 (12)	61·9±38·1 (21)

The numbers of subjects are shown in parentheses.

0·50%; $P>0·05$), when the results of the men up to the age of 60 years were compared with those from men over 60 years, the mean was significantly higher in the older group ($P<0·05$).

Relationships between age, clinical assessment of pubertal development and hormone measurements in children and adolescents

Age and clinical assessment (Table 3). The results have been related to the stage of genital development (Tanner, 1962). The age of these subjects in early puberty is older than expected, indicating a bias in this study—the subjects studied were mostly over the age of 14 years—thus boys commencing puberty at a later age than average were included in stages II and III. It can be seen that there was a progressive increase in pubic hair stage, testicular volume, height and weight with the successive stages of genital development. The heights and weights of the subjects with idiopathic growth retardation have not been included. The correlations between all these measurements were highly significant ($P<0·001$).

LH, FSH, testosterone and oestradiol levels. In Table 4 the blood levels of LH, FSH, testosterone and oestradiol have been grouped according to the stage of genital development of the subjects. Progressively higher mean levels of LH, testosterone and oestradiol were

TABLE 5. Correlation coefficients for LH, FSH, testosterone and oestradiol levels and clinical measurements in children and adolescents

	LH	FSH	Testosterone	Oestradiol
FSH	0·70***			
Testosterone	0·44**	0·29*		
Oestradiol	0·12	0·13	0·50***	
Age	0·61***	0·65***	0·69***	0·40**
Stage of genital development	0·73***	0·59***	0·82***	0·43**
Stage of pubic hair development	0·72***	0·58***	0·81***	0·40
Testicular volume	0·53***	0·12	0·71***	0·38*
Height	0·45***	0·28**	0·63***	0·46**
Weight	0·44***	0·28**	0·63***	0·50**

* $P<0·05$; ** $P<0·01$; *** $P<0·001$.

found with advancing genital development. FSH levels rose more abruptly at the onset of puberty (stage II).

Ten prepubertal boys had FSH levels at or below the limits of detectability of the FSH assays (0·16 and 0·10 iu/l) and the five very young children (1·7–4·0 years) had undetectable levels.

Subjects between the ages of 14 and 20 years who had achieved adult genital development (stage V) are included in Table 4. There were several points of interest in this group. Five subjects had FSH levels greater than 5·3 iu/l, and one had a level of 10·5 iu/l. Four of these results were confirmed in a second assay. There appeared to be no testicular or other abnormality in these boys in whom testicular volumes were between 18 and 25 ml. Testosterone and oestradiol levels were not measured in these subjects. When the results of the adolescents with adult genital development were compared with those of men aged between 21 and 50 years, the LH levels were significantly higher ($P<0·01$) and the testosterone levels significantly lower ($P<0·001$) in the younger subjects, but FSH levels and oestradiol levels were not significantly different. The relationships between hormone levels and the clinical measurements are shown in Table 5. There were highly significant correlations between LH, FSH and testosterone levels and age, stage of genital development, stage of pubic hair development, height and weight. The correlations of the latter with oestradiol levels were less significant and there was no correlation between testicular volume and FSH levels.

Plasma protein binding of testosterone and oestradiol. The binding capacities (q) of SHBG and the fractional and absolute free testosterone and oestradiol levels measured in the prepubertal boys and in plasma pools from pubertal subjects are shown in Table 6. The binding capacity was high in two prepubertal boys and in the normal adult male range after midpuberty. The fractional and absolute free steroid levels were low in the prepubertal subjects and were higher in samples from subjects with more advanced development.

Testicular volume secondary sex hair distribution and sexual function in adults

The frequencies of mean testicular volumes of 15 ml or less, reduced body hair and absent

TABLE 6. SHBG binding capacity (q) and free testosterone and oestradiol levels in boys before and during puberty. Stage I subjects aged 7 and 10 years, stages III and IV from plasma pools, stage V pools from subjects 14–16 and 17–19 years of age.

	Stage of genital development				
	I	II	III	IV	V
q ($\times 10^{-8}$ M)	15·0	—	6·2	4·5	5·4
	16·5				4·5
Free testosterone	0·22	—	0·93	1·60	1·51
(%)	0·32				1·39
(pmol/l)	6·9	—	125	233	399
Free oestradiol	0·61	—	2·44	2·84	2·82
(%)	0·44				2·61
(pmol/l)	0·22	—	0·26	0·85	2·41
	0·11				0·78

sexual function (impotence and/or lack of spontaneous erections and seminal emissions) in 20 year age groups are shown in Fig. 8.

Testicular volume. Two of the forty-two men in the 21–50 year age group had testicular volumes of 15 ml. Both appeared healthy, claimed normal sexual function and had no history of testicular disease. Neither was married and fertility was not proven. Because of the inaccuracy of the orchidometer and the termination of the scale at 25 ml, it was not possible to determine precise ranges of testicular volume in normal men and the dividing

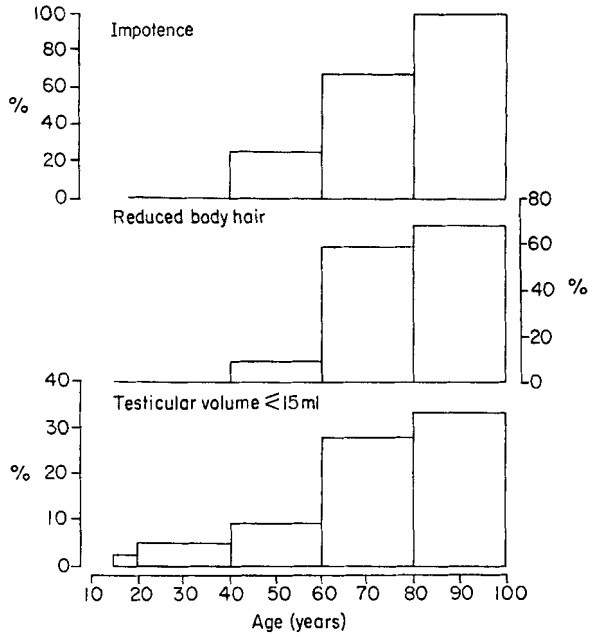


FIG. 8. Frequencies of impotence, reduced body hair and diminished testicular volume as a function of age in men.

line between normal sized and small testes has been taken to be 15 ml. In 15% of the adults studied, testicular volumes were greater than 25 ml; in these subjects testicular volume has been arbitrarily recorded as 30 ml for statistical analysis.

Mean testicular volumes of 15 ml or less were found in 27·5% of men over the age of 50 years and the frequency of small testes was significantly greater than in the 21–50 age group ($P<0{\cdot}01$) although the mean testicular volumes in the two groups were not significantly different. Some of the older men had recognizable testicular abnormalities; hydrocoele (three), unilateral atrophy following trauma (two) or epididymo-orchitis (one), and an absent right testis following hernia repair (two).

Body hair and sexual function. Secondary sex hair and sexual function were decreased in the majority of men over 60 years of age. Most of these men were patients in a geriatric hospital where opportunities for sexual expression are few. Eleven men aged between 70 and 101 years could not state precisely the time since their last erections or seminal emissions. Nineteen men aged between 70 and 96 years claimed they had not had erections or seminal emissions for periods ranging from 2 to 50 years. Three men aged 71, 74 and 81 years still

TABLE 7. Relationships between age and hormone measurements in men

Measurement	r	df	P
Plasma testosterone	−0·34	210	<0·001
Plasma oestradiol	0·18	178	<0·05
Serum LH	0·59	214	<0·001
Serum FSH	0·60	215	<0·001
q	0·57	60	<0·001
% Free testosterone	−0·52	48	<0·001
Free testosterone	−0·63	48	<0·001
% Free oestradiol	−0·49	50	<0·001
Free oestradiol	−0·42	47	<0·01
MCR^T (l/24 h)	−0·61	15	<0·01
MCR^T (l/24 h/m^2)	−0·53	15	<0·05
MCR^{E_2} (l/24 h)	−0·56	13	<0·05
MCR^{E_2} (l/24 h/m^2)	−0·50	13	<0·1
P_p^T	−0·34	15	NS
$P_p^{E_2}$	−0·10	13	NS
$P_{pp}^{TE_2}$	0·81	5	<0·05
Testicular volume	−0·19	81	<0·01
Reduced body hair score	0·83	134	<0·001
Reduced sexual function score	0·85	134	<0·001

df = number of pairs of observations − 2.

TABLE 8. Relationships between clinical and hormone measurements after exclusion of the effect of age

Relationship	r	df	P
LH–FSH	0·61	212	<0·001
LH–q	0·27	58	0·05
FSH–$P_p^{E_2}$	0·60	11	0·0–
% Free testosterone–% Free oestradiol	0·81	42	0·001
Free testosterone–Free oestradiol	0·34	39	0·05
Testicular volume–LH	0·37	77	0·001
Testicular volume–FSH	0·51	78	0·001
Testicular volume–testosterone	0·18	79	0·1
Reduced sexual function score–% Free testosterone	0·29	47	0·05

df = number of pairs of observations − 3.

had spontaneous erections with a frequency of at least one per week and the man aged 74 still enjoyed occasional sexual intercourse.

Relationships between age, hormone results and clinical assessment of virility

Effect of age. The correlation coefficients between age and the different clinical and hormone measurements are shown in Table 7. LH, FSH and oestradiol levels, the binding capacity of SHBG (q), $\rho_{pp}^{TE_2}$ and reduced body hair and impotence scores were positively

correlated with age. Testosterone, free testosterone and free oestradiol levels, MCR^T, MCR^{E_2} and testicular volume were negatively correlated with age.

Hormone and clinical measurements. There were many statistically significant correlations between the various hormone and clinical measurements but when the effect of age was eliminated by subtracting the age-correlated components, there were fewer significant relationships (Table 8). LH and FSH levels and free testosterone and oestradiol fractions were highly significantly correlated. There was a positive correlation between LH and q (the binding capacity of SHBG), a negative correlation between FSH and $P_p^{E_2}$ and both LH and FSH levels were inversely correlated with testicular volume.

The relationship between testicular volume and plasma testosterone levels was only significant at the 10% level. There was also an unexpected positive correlation between the per cent free testosterone levels and reduced sexual function. There were no significant relationships between the gonadotrophin and sex-steroid levels nor between the MCR results and the binding capacity of SHBG and free steroid levels after exclusion of the effect of age; this does not of course exclude the effect of age on SHBG causing the change in MCR^T. There was a significant inverse relationship between q and MCR^T ($P<0{\cdot}05$) but no relationship was seen between q and MCR^{E_2}.

DISCUSSION

Hormonal changes during puberty

Genital development. The close relationships between genital and pubic hair development, testicular volumes and blood levels of testosterone, LH and FSH found in this study generally confirm the results of Winter & Faiman (1972) and August *et al.* (1972).

Serum FSH and LH. Blood levels of FSH and LH rose from undetectable or low levels at the age of 8 years, and prior to the onset of puberty. There was a progressive rise in LH and a somewhat earlier, more abrupt and irregular rise in FSH levels during puberty. In a number of subjects with stage III–stage V genital development, and in more mature adolescents under the age of 20, LH levels were at or above the upper limit of the normal adult value.

Similar investigations of changes in gonadotrophin levels during childhood and adolescence have been reported, and the results have been reviewed by Root (1973) and Kulin & Reiter (1973). There is general agreement that LH and FSH levels rise prior to the onset of and during puberty in boys, but the magnitude and timing of changes with respect to the stages of development are controversial. Thus, Burr *et al.* (1970) reported that LH and FSH rose after the age of 9 years, that LH rose before FSH and the overall rise in FSH was greater than the rise in LH levels, whereas Winter & Faiman (1972) reported significant rises in both LH and FSH after the age of 6 years, and a more abrupt and greater rise in LH during puberty.

It must be noted that the hormone levels of single daytime samples in pubertal subjects should be interpreted cautiously in view of the occurrence of nocturnal episodes of LH and testosterone secretion which occur at the onset of puberty (Boyar *et al.*, 1972, 1974). This phenomenon does not, however, account for the high levels of LH and FSH in some pubertal boys and adolescents, and the higher mean levels in the mature subjects under the age of 20 compared to adult levels (21–50 years). There is no obvious explanation for this finding.

Testosterone and oestradiol. Testosterone levels were in the adult female range in prepubertal children and rose progressively with advancing pubertal development. Adult levels

were achieved only after completion of genital development, and thereafter levels increased up to the fourth decade. The values in prepubertal boys are slightly higher than those reported by others (Saez & Bertrand, 1968; Winter & Faiman, 1972; Boon *et al.*, 1972). The testosterone values in pubertal and young mature subjects are also different from those of Winter & Faiman (1972) who found no further changes in these levels after the age of 16 years. The lower mean testosterone and high LH levels in the mature teenagers compared with adults suggests that the setting of the feedback system may change during maturation, and that adult levels of these hormones may not be attained before the age of 20–25 years.

Oestradiol levels were low in prepubertal boys, and increased in the late stages of puberty.

Protein binding of testosterone and oestradiol. The binding capacity of SHBG and the free testosterone levels in children prior to and during puberty have been studied by Vermeulen *et al.* (1969, 1972). The values obtained in this present limited study, which showed that the binding capacity of SHBG was high before puberty and fell to adult levels before the completion of pubertal development, are comparable. The finding of adult male binding capacities in plasma pools from subjects at different stages of genital development, and of testosterone levels below the normal range for men in the same pools, indicate that the mechanism which controls the elaboration of SHBG is sensitive to relatively low circulating testosterone levels.

Hormonal changes with age in the adult

Serum FSH and LH. Comparison of results of the present study with ranges of serum LH and FSH reported by other investigators is difficult because of changes which occur with age, and because different assay systems have been used. Mean levels for healthy young men measured by bioassay or radioimmunoassay in terms of the second IRP, HMG vary between 6·1 and 24·2 iu/l for LH; and between 4·1 and 16·9 iu/l for FSH (Burger *et al.*, 1972). The variability between these results has been attributed to differences in the antisera and techniques employed. In this study lower ranges for LH and FSH probably result from the use of a pituitary gonadotrophin preparation for standards. However, even when the same reagents are used in different laboratories the results may not be identical (Bangham *et al.*, 1973).

Ryan *et al.* (1970) have recognized that values obtained by single estimates of LH and FSH in men aged between 20 and 49 years are not normally distributed. Our data for LH and FSH in most age groups was distributed in a lognormal fashion. However, when the results from men over the age of 70 years were included, logarithmic transformation did not completely correct for the skew towards higher levels.

The results of this present investigation confirm and extend a number of other studies. Schalch *et al.* (1968) demonstrated that LH levels were significantly higher in a group of old men than in normal young men. Ryan & Faiman (1968) found that both LH and FSH levels were significantly elevated in men over the age of 50 years compared with levels in men aged between 20 and 49 years. Because not all old men have high levels, they suggest that there are two populations with either normal or high levels. Similar results were found by Burger *et al.* (1972), Stearns *et al.* (1974) and Rubens *et al.* (1974). Rises in LH levels without significant changes in FSH levels and the reverse, a significant rise in FSH but not LH levels with age, have also been claimed (Swerdloff & Odell, 1968; Wide *et al.*, 1973).

We have shown that both LH and FSH levels are elevated in a proportion of elderly men, and also that there is a gradual and progressive rise in the levels of both these hormones,

TABLE 9. Reported values for plasma oestradiol in healthy men.

Authors	Method	Mean (pmol/l)	Range (pmol/l)	No. of subjects
Baird & Guevara (1969)	DID	96·3	UD–215	7
Korenman *et al.* (1969)	UC	88·9	41–137	6
Abraham & Odell (1970)	RIA	66·7	—	pool
Mayes & Nugent (1970)	CPB	111·1	30–170	15
Mikhail *et al.* (1970)	RIA	63·0	44–81	6
Nagai & Longcope (1971)	UC	96·3	44–144	20
Jenner *et al.* (1972)	RIA	66·7	30–104	—
Saez *et al.* (1972)	RIA	100	63–137	10
Kent *et al.* (1973)	RIA	59·3	22–133	12
Galvao-Teles *et al.* (1973)	CPB	85·2	UD–211	—
Chopra *et al.* (1973)	RIA	100	63–137	12
Doerr & Pirke (1973)	RIA	63·0	37–104	51
Rubens *et al.* (1974)	RIA	51·8 (< 50 years) 77·8 (> 50 years)		
Present study	RIA	59·3	UD–126	119

The methods used were double isotope derivative (DID), uterine cytosol binding protein (UC), competitive protein binding (CPB), or radioimmunoassay (RIA).

UD, results below sensitivity of method.

which commences about the age of 40 years. There was also a highly significant correlation between LH and FSH levels. The failure of other studies to demonstrate similar changes uniformly may be due to subject selection. In this study, many of the older men were patients in a geriatric hospital, and different results might be obtained in elderly men selected on the basis of the absence of any obvious illness.

Plasma testosterone. The range of testosterone levels in men aged between 21 and 50 years, 11·8–37·5 nmol/l, is in agreement with ranges reported by others using a variety of methods. Although several authors claim that plasma testosterone levels remain within the same range from adolescence until old age, the numbers of subjects studied has been small and inspection of the data reveals a trend toward lower levels in older men (Coppage & Cooner, 1965; Kent & Acone, 1966; Hudson *et al.*, 1967; Gandy & Peterson, 1968; Robinson & Thomas, 1971). Kirschner & Coffman (1968) found significantly lower test-

TABLE 10. Mean binding capacity of SHBG ($\times 10^{-8}$ M) in healthy subjects

Authors	Men 20–50 years	Women 20–35 years	Prepubertal children	Pregnancy
Vermeulen *et al.* (1969, 1971)	4·6, 5·2	7·4	7·1	28·5
Corvol *et al.* (1971)	1·7	4·9		37·5
Rosenfeld (1971)	3·4	6·5	12·4	
Rosner (1972)	3·2	6·4		39·0
Burke & Anderson (1972)	3·9	7·8		
Galvao-Teles *et al.* (1973)	4·9			
Rudd *et al.* (1974)	5·0	7·8	7·0	29·0

osterone levels in six men aged between 55 and 65 years compared with levels in men aged 18–38 years. Vermeulen *et al.* (1972) and Rubens *et al.* (1974) studied changes in testosterone secretion and metabolism with age in greater detail, and demonstrated a significant fall in plasma testosterone levels after the age of 60 years. The pattern seen was very similar to that shown in Fig. 3, although a number of their old men had levels below 6·9 nmol/l. Similar results have been reported by Nieschlag *et al.* (1973), Doerr & Pirke (1973) and Stearns *et al.* (1974).

Plasma oestradiol. Since only small numbers of normal individuals have been studied by most investigators, the range of values for plasma oestradiol is not so well established (Table 9). In this study, the mean and range of values were 59, and from 2·9 to 126 pmol/l, which are similar to those of Doerr (1973) and to most other values found using radioligand methods, not many of which have studied changes in plasma oestradiol with age.

In this study there was a small but significant difference in the plasma oestradiol levels between young men and old men. This confirms the study of Doerr & Pirke (1973) who also found oestradiol levels to be higher in older men. On the other hand, Nagai & Longcope (1971) and Longcope (1973) found no differences in one study and lower values in older men in the other.

SHBG binding capacity. The mean binding capacity of SHBG ($4{\cdot}3 \pm 1{\cdot}2 \times 10^{-8}$ M) falls within the range of values reported by others for healthy young men (Table 10). Our data indicate higher SHBG capacities in older men, which is in agreement with other studies (Vermeulen *et al.*, 1972; Doerr & Pirke, 1973).

Free testosterone and oestradiol levels. The mean free testosterone level, $0{\cdot}38 \pm 0{\cdot}17$ nmol/l, is also in close agreement with values calculated by Vermeulen *et al.* (1971) and Baulieu *et al.* (1971). The free testosterone levels in different age groups were almost identical to those reported by Vermeulen *et al.* (1971, 1972). Increased plasma protein binding of testosterone in older men was also found by Stearns *et al.* (1974) and Nieschlag *et al.* (1973).

The mean free oestradiol level (1·44 pmol/l) found in this study is in reasonable agreement with those found by others (Chopra *et al.*, 1973; Galvao-Teles *et al.*, 1973; Rubens *et al.*, 1974). Changes in the levels of free oestradiol with age have been reported by Rubens *et al.* (1974) who found no significant differences in these levels between young and old men. In this study free oestradiol levels were inversely correlated with age, and there was a significant difference between men under and over the age of 50 ($P<0{\cdot}05$).

Metabolic clearance rates of testosterone and oestradiol. The values found in this group of men, notwithstanding the small numbers, are consistent with other studies. For the younger men the mean MCR^T of 1137 ± 213 l/24 h is very similar to the 1190 ± 231 l/24 h reported by Southern *et al.* (1973) from thirty-one normal men. The plasma production rates of testosterone for these younger men (13·5–44·1 μmol/24 h) fall within the accepted range of 10·4–45·1 μmol/24 h (Hudson *et al.*, 1970). Significant reductions in MCR^T and P_p^T with age are also consistent with other studies (Kent & Acone, 1966; Isurugi, 1967; Vermeulen *et al.*, 1972; Bayard *et al.*, 1973).

The previously reported range for MCR^{E_2} was 1240–2610 l/24 h (Longcope & Tait, 1971; Hembree *et al.*, 1969; Ruder *et al.*, 1971). The range for the MCR^{E_2} in this study was slightly greater; 1189–3069 l/24 h with a mean of 1804 l/24 h, which is similar to that reported by Longcope *et al.* (1968). Although the MCR^{E_2} was measured in only four men over the age of 50 years, the mean value, 1076 l/24 h was significantly less than the mean value found in the eleven men from the younger age group.

The $P_p^{E_2}$ values were widely scattered and were not obviously affected by the age of the subjects and the difference in the two age groups was not significant. The values are similar to oestradiol production rates determined by the urinary specific activity technique (Eren *et al.*, 1967) but only half the mean value for healthy men determined by Ruder *et al.* (1971) using measurements of MCR^{E_2} and plasma oestradiol levels.

Interconversion of testosterone and oestradiol. Transfer constants for the peripheral conversion of testosterone to oestradiol in three men under the age of 50 were lower than those reported by Longcope *et al.* (1969), although the conversion ratios were in close agreement, and the means (0·18%) were identical. MacDonald *et al.* (1967, 1971) used urinary measurements to determine transfer constants and have reported values of 0·3 and 0·4%. They have also stated that transfer constants for the peripheral conversion of testosterone to oestradiol and of androstenedione to estrone increase with age but have not presented data to support this statement. Because only a small number of subjects was included in our study, and as the results in the young men are somewhat lower than those reported by Longcope *et al.* (1969), the finding of a positive correlation between $\rho_{pp}^{TE_2}$ and age requires confirmation. However, two of the four subjects over 50 years of age had values above the highest found by Longcope *et al.* (1969); an elevated $\rho_{pp}^{TE_2}$ appears to be an accompaniment of androgen deficiency (Baker *et al.*, 1976). Therefore this finding is unlikely to be the result of a quirk of sampling.

Seasonal effects on plasma FSH, LH, testosterone and oestradiol. Seasonal variations in reproductive function are frequently observed in lower animals. In dogs there is increased testosterone secretion in the spring (Eik-Nes, 1971), while in the rhesus monkey in captivity the secretion of testosterone is higher in the autumn and winter (Michael *et al.*, 1975; Robinson *et al.*, 1975). In a previous study of six healthy young men, testosterone and LH levels were higher in the spring than in the winter (Burger *et al.*, 1972). In this present study in which observations were made on twenty-six subjects, the LH, FSH and testosterone levels were lower in the winter, but the differences between the seasons were small and not significant.

Overall conclusions

These results confirm that testicular function declines with age in men (see also Editorial, 1975). Unlike the menopause in women, this degeneration does not appear to occur regularly, consistently or suddenly at any particular age. The elevated LH and FSH levels suggest that the mechanisms of senescence operate primarily at a testicular level. Longcope (1973) and Rubens *et al.* (1974) studied the effect of HCG administration in young and old men. There were significantly greater absolute rises in testosterone and oestradiol levels in the younger subjects, but the relative responses of the pretreatment levels were not significantly different in the two age groups. Similar results have also been found by Frick & Kincl (1969) in old men with histological evidence of testicular degeneration. Longcope interpreted these results as a reduction in pituitary gonadotrophin secretion in elderly men. The present results suggest that the secretion of LH and FSH is actually increased in old age.

Possible mechanisms for a senescent testicular degeneration include a progressive depletion of germinal elements with age, cumulative minor traumatic lesions and vascular insufficiency. If there is a depletion of germinal cell elements, how does the Leydig cell dysfunction arise? The fact that not all the older men with elevated gonadotrophins had clinical evidence of testicular atrophy suggests that senescent changes in the testis may be quite subtle.

Whatever may be the underlying mechanisms, we would propose the following sequence of events. Germinal epithelial damage leads to elevated FSH levels; Leydig cell damage leads to reduced testosterone secretion, low circulating levels of testosterone and a low metabolic clearance rate with elevation of LH levels and loss of virility. Examination of the relationships between clinical and hormone measurements, after excluding the effects of age, supports this sequence. Elevated LH and FSH levels were related to testicular atrophy, and there was a trend for low testosterone levels to be associated with small testes. However, the expected correlations between indices of testosterone production and virility were not statistically significant, suggesting the interplay of other factors such as psychic aspects of sexual function and senescent changes in hair follicles.

Although this sequence of events would appear to be reasonable, explanations of changes in SHBG, oestradiol secretion and $\rho_{pp}^{TE_2}$ must remain more speculative. Because correlations between q and testosterone and oestradiol levels were not statistically significant after excluding the effect of age, it cannot be stated whether testosterone or oestradiol controls SHBG levels. The correlation between q and LH levels is compatible with the suggestion that testosterone not bound to SHBG is active in the feedback control of LH (Ruder *et al.*, 1971; Anderson, 1974). The inverse relationship between FSH levels and $P_p^{E_2}$ may indicate a role for oestradiol in the control of FSH levels in the male, but this is tenuous because there was no significant correlation between plasma oestradiol and FSH levels.

The combination of reduced P_p^T increased $\rho_{pp}^{TE_2}$ and maintained $P_p^{E_2}$ with age is interesting. The actual mass of oestradiol resulting from the higher peripheral conversion of testosterone to oestradiol in the older men does not appear to be increased because of the reduced P_p^T. It is possible that other peripheral conversions such as that of androstenedione to oestrone, and of oestrone to oestradiol, may be increased and contribute to the maintenance of oestradiol production in old age. Another possibility is that there is a change in the ratio of testosterone and oestradiol secreted by the testes. Doerr & Pirke (1973) found a significant correlation between testosterone and oestradiol levels in men which might be expected from simultaneous testicular secretion of testosterone and oestradiol and the peripheral conversion of testosterone to oestradiol. On the other hand, Johnsen *et al.* (1971) have produced evidence that testosterone and oestradiol are produced by different compartments in the testis. This proposition is further supported by the observation that Sertoli cells synthesize oestradiol from testosterone when grown in cell culture, and that this reaction is stimulated by FSH (Dorrington & Armstrong, 1975). The lack of correlation between testosterone and oestradiol levels in the present study also suggests that oestradiol and testosterone secretion by the testis may occur independently. Thus the total plasma production rate of oestradiol may be maintained in old age by a combination of increased peripheral conversion of precursors, and also by a greater secretion of oestradiol by the testes relative to the waning secretion of testosterone.

The significance of the continuing production of oestradiol in the face of reduced testosterone production and reduced MCR^{E_2} resulting in elevated circulating levels of oestradiol is obscure. Could the increase in oestradiol lead to the high SHBG levels and therefore tend to conserve testosterone for action in target tissues by protecting it from degradation? Vermeulen *et al.* (1972) found that there was a shift in the pattern of testosterone metabolism in the elderly with a relative increase in 5β-reduction, and decreased androstenediol formation by the 17β-hydroxy pathway, indicating there may be reduced testosterone metabolism in target tissues, and therefore probably reduced testosterone action in old men. They

also found a positive correlation between the free testosterone fraction and the amount of 5α-androstane-3α,17β-diol excreted in the urine, which suggests that testosterone bound to SHBG is relatively unavailable to target tissues. Thus, it would appear that the elevated SHBG capacity in older men would serve not as a mechanism for maintaining androgenicity but with decreased testosterone secretion and increased peripheral conversion of testosterone further to limit the activity and biological availability of testosterone.

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REFERENCES

ABRAHAM, G.E. & ODELL, W.D. (1970) Solid-phase radioimmunoassay of serum estradiol 17β: a semi-automated approach. *Immunologic Methods in Steroid Determination* (ed. by F. G. Peron and B. V. Caldwell), pp. 87–112. Meredith Corporation, New York.

ALBERT, A. (1956) Human urinary gonadotropin. *Recent Progress in Hormone Research*, **12**, 227–301.

ALFORD, F.P., BAKER, H.W.G., BURGER, H.G., DE KRETSER, D.M., HUDSON, B., JOHNS, M.W., MASTERTON, J.P., PATEL, Y.C. & RENNIE, G.C. (1973) Temporal patterns of integrated hormone levels during sleep and wakefulness. II. Follicle stimulating hormone, luteinising hormone, testosterone and estradiol. *Journal of Clinical Endocrinology and Metabolism*, **37**, 848–854.

ANDERSON, D.C. (1974) Sex hormone binding globulin. *Clinical Endocrinology*, **3**, 69–96.

AUGUST, G.P., GRUMBACH, M.M. & KAPLAN, S.I. (1972) Hormonal changes in puberty. III. Correlation of plasma testosterone, LH, FSH, testicular size, and bone age with male pubertal development. *Journal of Clinical Endocrinology and Metabolism*, **34**, 319–326.

BAIRD, D.T. & GUEVARA, A. (1969) Concentration of unconjugated estrone and estradiol in peripheral plasma in nonpregnant women, throughout the menstrual cycle, castrate and postmenopausal women and in men. *Journal of Clinical Endocrinology and Metabolism*, **29**, 149–156.

BAKER, H.W.G., BURGER, H.G., DE KRETSER, D.M., DULMANIS, A., HUDSON, B., O'CONNOR, S., PAULSEN, C.A., PURCELL, N., RENNIE, G.C., SEAH, C.S., TAFT, H.P. & WANG, C. (1976) A study of the endocrine manifestations of hepatic cirrhosis. *Quarterly Journal of Medicine*, **45**, 145–178.

BAKER, H.W.G., BURGER, H.G., DE KRETSER, D.M., HUDSON, B., STRAFFON, W.G.E. (1973) Effects of synthetic oral oestrogens in normal men and patients with prostatic carcinoma: lack of gonadotrophin suppression by chlorotrianisene. *Clinical Endocrinology*, **2**, 297–306.

BANGHAM, D.R., BERRYMAN, I., BURGER, H.G., COTES, P.M., FURNIVAL, B.E., HUNTER, W.M., MIDGLEY, A.R., MUSSETT, M.V., REICHERT, L.E., ROSEMBERG, E., RYAN, R.J. & WIDE, L. (1973) An international collaborative study of 69/104, a reference preparation of human pituitary FSH and LH. *Journal of Clinical Endocrinology and Metabolism*, **36**, 647–660.

BAULIEU, E.E., ALBERGA, A., JUNG, I., LEBEAU, M.C., MERCIER-BODAID, C., MILGROM, E., REYNAUD, J.P., REYNAUD-JAMMET, C., ROCHEFORT, H., TRUONG, H. & ROBEL, P. (1971) Metabolism and protein binding of sex steroids in target organs: An approach to the mechanism of hormone action. *Recent Progress in Hormone Research*, **27**, 351–419.

BAYARD, F., LOUVET, J.P., THIJSSENS, J.L., THOUYENOT, J.P. & BOULARD, C. (1973) Testosterone and 17β-oestradiol production rate in the ageing man. (Abstract). *Acta Endocrinologica* Suppl., 177, 121.

BENNETT, H., BAGGENSTOSS, A. & BUTT, H. (1950) The testis, breast and prostate of men who die of cirrhosis of the liver. *American Journal of Clinical Pathology*, **20**, 814–828.

BOON, D.A., KEENAN, R.E., SLAUNWHITE, W.R. & ACETO, T. (1972) Conjugated and unconjugated plasma androgens in normal children. *Pediatric Research*, **6**, 111–118.

BOYAR, R., FINKELSTEIN, J., ROFFWARG, H., KAPEN, S., WEITZMAN, E. & HELLMAN, L. (1972) Synchronization of augmented luteinizing hormone secretion with sleep during puberty. *New England Journal of Medicine*, **287**, 582–586.

BOYAR, R.M., ROSENFELD, R.S., KAPEN, S., FINKELSTEIN, J.W., ROFFWARG, H.P., WEITZMAN, E.D. & HELLMAN, L. (1974) Simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. *Journal of Clinical Investigation*, **54**, 609–618.

BURGER, H.G., BAKER, H.W.G., HUDSON, B. & TAFT, H.P. (1972) Gonadotropic and gonadal function in the normal adult male. *Gonadotropins* (ed. by B. B. Saxena, C. G. Beling and H. M. Gandy), pp. 569–592. Wiley Interscience, New York.

BURKE, C.W. & ANDERSON, D.C. (1972) Sex-hormone-binding globulin as an oestrogen amplifier. *Nature*, **240**, 38–40.

BURR, I.M., SIZONENKO, P.C., KAPLAN, S.L. & GRUMBACH, M.M. (1970) Hormonal changes in puberty. I. Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty, testicular size and bone age in normal boys. *Pediatric Research*, **4**, 25–35.

CHOPRA, I.J., TULCHINSKY, D. & GREENWAY, F.L. (1973) Estrogen–androgen imbalance in hepatic cirrhosis. *Annals of Internal Medicine*, **79**, 198–203.

COPPAGE, W.S., JR & COONER, A.E. (1965) Testosterone in human plasma. *New England Journal of Medicine*, **273**, 902–907.

CORVOL, P.L., CHRAMBACH, A., RODBARD, D. & BARDIN, C.W. (1971) Physical properties and binding capacity of testosterone–estradiol-binding globulin in human plasma, determined by polyacrylamide gel electrophoresis. *Journal of Biological Chemistry*, **246**, 3435–3443.

DOERR, P. (1973) Hapten-radioimmunoassay of plasma oestradiol. *Acta Endocrinologica*, Suppl., **72**, 330–344.

DOERR, P. & PIRKE, K.M. (1973) Influence of male senescence on plasma oestradiol, testosterone and binding capacity of testosterone-binding globulin. (Abstract). *Acta Endocrinologica*, Suppl., **177**, 123.

DORRINGTON, J.H. & ARMSTRONG, D.T. (1975) Follicle-stimulating hormone stimulates estradiol-17β synthesis in cultured Sertoli cells. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 2677–2681.

DUFAU, M.L., DULMANIS, A., CATT, K.J. & HUDSON, B. (1970) Measurement of plasma estradiol-17β by competitive protein binding assay employing pregnancy plasma. *Journal of Clinical Endocrinology and Metabolism*, **30**, 351–356.

EDITORIAL (1975) Hormones and elderly testes. *British Medical Journal*, **iii**, 2–3.

EIK-NES, K.B. (1971) Production and secretion of testicular steroids. *Recent Progress in Hormone Research*, **27**, 517–535.

ENGLE, E.T. & PINCUS, G. (1956) *Hormones and the Ageing Process*, pp. 1–20. Academic Press, New York.

EREN, S., REYNOLDS, G.H., TURNER, M.E., JR, SCHMIDT, F., MACKAY, J.H., HOWARD, C.M. & PREEDY, J.R.K. (1967) Estrogen metabolism in the human. II. Studies in the male using estradiol-17β-6,7-^{3}H. *Journal of Clinical Endocrinology and Metabolism*, **27**, 819–927.

FRICK, J. & KINCL, F.A. (1969) The measurement of plasma testosterone by competitive protein binding assay. *Steroids*, **13**, 495–505.

GALVAO-TELES, A., ANDERSON, D.C., BURKE, C.W., MARSHALL, J.C., CORKER, C.S., BROWN, R.L. & CLARK, M.L. (1973) Biologically active androgens and oestradiol in men with chronic liver disease. *Lancet*, **i**, 173–177.

GANDY, H.M. & PETERSON, R.E. (1968) Measurement of testosterone and 17-keto-steroids in plasma by the double isotope dilution derivative technique. *Journal of Clinical Endocrinology and Metabolism*, **28**, 949–977.

HAMILTON, J.B. (1951) Quantitative measurement of a secondary sex character, axillary hair. *Annals of the New York Academy of Sciences*, **53**, 585–599.

HEMBREE, W.C., BARDIN, C.W. & LIPSETT, M.B. (1969) A study of estrogen metabolic clearance rates and transfer factors. *Journal of Clinical Investigation*, **48**, 1809–1819.

HENDERSON, W.R. & ROWLANDS, I.W. (1938) Gonadotropic activity of anterior pituitary gland in relation to increased intracranial pressure. *British Medical Journal*, **i**, 1094–1097.

HOLLANDER, N. & HOLLANDER, V.P. (1958) The microdetermination of testosterone in human spermatic vein blood. *Journal of Clinical Endocrinology and Metabolism*, **18**, 966–971.

HORTON, R. & TAIT, J.F. (1966) Androstenedione production and interconversion rates measured in peripheral blood and studies of the possible site of its conversion to testosterone. *Journal of Clinical Investigation*, **45**, 305–313.

HUDSON, B., BURGER, H.G., DE KRETSER, D.M., COGHLAN, J.P. & TAFT, H.P. (1970) Testosterone plasma levels in normal and pathological conditions. *The Human Testis* (ed. by E. Rosemberg and C. A. Paulsen), pp. 423–437. Plenum Press, New York.

HUDSON, B., COGHLAN, J.P. & DULMANIS, A. (1967) Testicular function in man. *Endocrinology of the Testis, Ciba Foundation Colloquia on Endocrinology*, Vol. 16 (ed. by G. E. W. Wolstenholme and M. O'Connor), pp. 140–153. Churchill, London.

HUDSON, B., COGHLAN, J.P., DULMANIS, A. & WINTOUR, M. (1965) The measurement of testosterone in biological fluids in the evaluation of androgen activity. *Proc. 2nd Int. Congr., Endocr., London*, 1965, pp. 1127–1133. Int. Congr. Series No. 83, Excerpta Medica Foundation, Amsterdam.

ISURUGI, K. (1967) Plasma testosterone production rates in patients with prostatic carcinoma and benign prostatic hypertrophy. *Journal of Urology*, **97**, 903–908.

JENNER, M.R., KELCH, R.P., KAPLAN, S.L. & GRUMBACH, M.M. (1972) Hormonal changes in puberty. VI. Plasma estradiol, LH and FSH in prepubertal children, pubertal females, and in precocious puberty, premature thelarche, hypogonadism, and in a child with a feminizing ovarian tumour. *Journal of Clinical Endocrinology and Metabolism*, **34**, 521–530.

JOHNSEN, S.G. (1959) A clinical routine-method for the quantitative determination of gonadotrophins in 24-hour urine samples. II. Normal values for men and women at all age groups from puberty to senescence. *Acta Endocrinologica*, **34**, 209–227.

JOHNSEN, S.G., CHRISTIANSEN, P., FRANDSEN, A., FRØLAND, A. & NIELSEN, J. (1971) Analysis of a dexamethasone/chorionic gonadotrophin test of adrenogonadal function in normal men as compared with dexamethasone test in normal women. *Acta Endocrinologica*, **66**, 587–605.

KENT, J.R. & ACONE, A.B. (1966) Plasma testosterone levels and ageing in males. *Androgens in Normal and Pathological Conditions, Int. Congr. Series* No. 101 (ed. by A. Vermeulen and D. Exley), pp. 31–35. Excerpta Medica Foundation, Amsterdam.

KENT, J.R., SCARAMUZZI, R.J., LAUWERS, W., PARLOW, A.F., HILL, M., PENARDI, R. & HILLIARD, J. (1973) Plasma testosterone, estradiol, and gonadotrophins in hepatic insufficiency. *Gastroenterology*, **64**, 111–115.

KINSEY, A.C., POMEROY, W.B. & MARTIN, C.E. (1948) *Sexual Behaviour in the Human Male*, p. 226. Saunders, Philadelphia.

KIRSCHNER, M.A. & COFFMAN, G.D. (1968) Measurement of plasma testosterone and Δ^4-androstenedione using electron capture gas-liquid chromatography. *Journal of Clinical Endocrinology and Metabolism*, **28**, 1347–1355.

KORENMAN, S.G., PERRIN, L.E. & MCCALLUM, T.P. (1969) A radio-ligand binding assay system for oestradiol measurement in human plasma. *Journal of Clinical Endocrinology and Metabolism*, **29**, 879–883.

KULIN, H.E. & REITER, W.E. (1973) Gonadotropins during childhood and adolescence: a review. *Pediatrics*, **51**, 260–271.

LONGCOPE, C. (1973) The effect of human chorionic gonadotropin in plasma steroid levels in young and old men. *Steroids*, **21**, 583–590.

LONGCOPE, C., KATO, T. & HORTON, R. (1969) Conversion of blood androgens to estrogens in normal adult men and women. *Journal of Clinical Investigation*, **48**, 2191–2201.

LONGCOPE, C., LAYNE, D.W. & TAIT, J.F. (1968) Metabolic clearance rates and interconversion of oestrone and 17β estradiol in normal males and females. *Journal of Clinical Investigation*, **47**, 93–106.

LONGCOPE, C. & TAIT, J.F. (1971) Validity of metabolic clearance and interconversion rates of estrone and 17β-estradiol in normal adults. *Journal of Clinical Endocrinology and Metabolism*, **32**, 481–490.

MacDonald, P.C., Grodin, J.M. & Siiteri, P.K. (1971) Dynamics of androgen and oestrogen secretion, *Control of Gonadal Steroid Secretion* (ed. by D. T. Baird and J. A. Strong), pp. 158–174. Edinburgh University Press.

MacDonald, P.C., Rombaut, R.P. & Siiteri, P.K. (1967) Plasma precursors of estrogen. I. Extent of conversion of plasma Δ^4-androstenedione to estrone in normal males and nonpregnant, normal, castrate and adrenalectomized females. *Journal of Clinical Endocrinology and Metabolism*, **27**, 1103–1111.

Mayes, D. & Nugent, C.A. (1970) Plasma estradiol determined with a competitive protein binding method. *Steroids*, **15**, 389–403.

Melick, R.A. & Taft, H.P. (1959) Observations on body hair in old people. *Journal of Endocrinology and Metabolism*, **19**, 1597–1607.

Michael, R.P., Zympe, D., Plant, T.M. & Evans, R.G. (1975) Annual changes in the sexual potency of captive male rhesus monkeys. *Journal of Reproduction and Fertility*, **45**, 169–172.

Mikhail, G., Wu, C.H., Ferin, M. & Vande Wiele, R.L. (1970) Radioimmunoassay of plasma estrone and estradiol. *Steroids*, **15**, 333–352.

Morer-Fargas, F. & Nowakowski, H. (1965) Die Testosteronausscheidung im Harn bei mannlichen Individuen. *Acta Endocrinologica*, **49**, 443–452.

Nagai, N. & Longcope, C. (1971) Estradiol-17β and estrone: studies on their binding to rabbit uterine cytosol and their concentration in plasma. *Steroids*, **17**, 631–647.

Nieschlag, E., Kley, K.H. & Wiegelmann, W. (1973) Age dependence of the endocrine testicular function in adult men. (Abstract). *Acta Endocrinologica* Suppl., 177, 122.

O'Connor, S., Baker, H.W.G., Dulmanis, A. & Hudson, B. (1973) The measurement of sex steroid binding globulin by differential ammonium sulphate precipitation. *Journal of Steroid Biochemistry*, **4**, 331–339.

Prader, A. (1966) Testicular size: assessment and clinical importance. *Triangle*, **7**, 240–243.

Robinson, J.A., Scheffler, G., Eiselle, S.G. & Goy, R.W. (1975) Effects of age and season on sexual behaviour and plasma testosterone and dihydrotestosterone concentrations of laboratory housed male rhesus monkeys (*Macaca mulatta*). *Biology of Reproduction*, **13**, 203–210.

Robinson, M.R.G. & Thomas, B.S. (1971) Effect of hormonal therapy on plasma testosterone levels in prostatic carcinoma. *British Medical Journal*, **iv**, 391–394.

Root, A.W. (1973) Endocrinology of puberty. I. Normal sexual maturation. *Journal of Pediatrics*, **83**, 1–19.

Rosenfeld, R.L. (1971) Plasma testosterone binding globulin and indexes of the concentration of unbound plasma androgens in normal and hirsute subjects. *Journal of Clinical Endocrinology and Metabolism*, **32**, 717–728.

Rosner, W. (1972) A simplified method for the quantitative determination of testosterone-estradiol-binding globulin activity in human plasma. *Journal of Clinical Endocrinology and Metabolism*, **34**, 983–988.

Rubens, R., Dhowt, M. & Vermeulen, A. (1974) Further studies on Leydig cell function in old age. *Journal of Clinical Endocrinology and Metabolism*, **39**, 40–45.

Rudd, B.T., Duignan, N.M. & London, D.R. (1974) A rapid method for the measurement of sex hormone binding globulin capacity of sera. *Clinica Chimica Acta*, **55**, 165–178.

Ruder, H., Corvol, P., Mahoudeau, J.A., Ross, G.T. & Lipsett, M.B. (1971) Effects of induced hyperthyroidism on steroid metabolism in man. *Journal of Clinical Endocrinology and Metabolism*, **33**, 382–387.

Ryan, R.J., Cloutier, M.D., Hayles, A.B., Paris, J. & Randall, R. (1970) The clinical utility of radioimmunoassays for serum follicle stimulating hormone (FSH) and luteinizing hormone (LH). *Medical Clinics of North America*, **54**, 1049–1059.

Ryan, R.J. & Faiman, C. (1968) Radioimmunoassay of FSH and LH in human serum: the effects of age and infusion of several polyamines in males. *Gonadotropins* (ed. by E. Rosemberg), pp. 333–337. Geron-X Inc., Los Altos, California.

Saez, J.M. & Bertrand, J. (1968) Studies on testicular function in children: plasma concentrations of testosterone, dehydroepiandrosterone and its sulfate before and after stimulation with human chorionic gonadotrophin. *Steroids*, **12**, 749–761.

Saez, J.M., Morera, A.M., Dazord, A. & Bertrand, J. (1972) Adrenal and testicular contribution to plasma oestrogens. *Journal of Endocrinology*, **55**, 41–49.

Schalch, D.W., Parlow, A.F., Boon, R.C., Reichlin, S. & Lee, L.A. (1968) Measurement of human luteinizing hormone in plasma by radioimmunoassay. *Journal of Clinical Investigation*, **47**, 665–678.

Snedecor, G.W. & Cochran, W.G. (1967) *Statistical Methods*, 6th edn. Iowa State University Press, Ames, Iowa.

SOUTHREN, A.L., GORDON, G.G., OLIVO, J., RAFII, F. & ROSENTHAL, W.S. (1973) Androgen metabolism in cirrhosis of the liver. *Metabolism*, **22**, 695–702.

STEARNS, E.L., MACDONELL, J.A., KAUFMAN, B.J., PADUA, R., LUCMAN, T.S., WINTER, J.S.D. & FAIMAN, C. (1974) Declining testicular function with age: hormonal and clinical correlates. *American Journal of Medicine*, **57**, 761–766.

SWERDLOFF, R.S. & ODELL, W.D. (1968) Some aspects of the control of secretion of LH and FSH in humans. *Gonadotropins* (ed. by E. Rosemberg), pp. 155–166. Geron-X Inc., Los Altos, California.

TANNER, J.M. (1962) *Growth at Adolescence*. Blackwell Scientific Publications, Oxford.

VERMEULEN, A., RUBENS, R. & VERDONCK, L. (1972) Testosterone secretion and metabolism in male senescence. *Journal of Clinical Endocrinology and Metabolism*, **34**, 730–735.

VERMEULEN, A., STOICA, T. & VERDONCK, L. (1971) The apparent free testosterone concentration, an index of androgenicity. *Journal of Clinical Endocrinology and Metabolism*, **33**, 759–767.

VERMEULEN, A., VERDONCK, L., VAN DER STRAETEN, M. & ORIE, N. (1969) Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate. *Journal of Clinical Endocrinology and Metabolism*, **29**, 1470–1480.

WANG, C., YOUATT, G., O'CONNOR, S., DULMANIS, A. & HUDSON, B. (1974) Simple radioimmunoassay for plasma testosterone plus 5α-dihydrotestosterone. *Journal of Steroid Biochemistry*, **5**, 551–555.

WIDE, L., NILLIUS, S.J., GEMZELL, C. & ROOS, P. (1973) Radioimmunosorbent assay of follicle-stimulating hormone and luteinizing hormone in serum and urine from men and women. *Acta Endocrinologica* Suppl., **174**, 1–58.

WINTER, J.S.D. & FAIMAN, C. (1972) Pituitary–gonadal relations in male children and adolescents. *Pediatric Research*, **6**, 126–135.