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IV. CLINICAL ENDOCRINOLOGY

Klinefelter's Syndrome and Its Variants: A Hormonal and Chromosomal Study[1,2]

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I. Introduction

In 1942 Klinefelter, Reifenstein, and Albright described the features of a new testicular disorder. This syndrome was characterized by the presence of gynecomastia, azoospermia, varying degrees of Leydig cell function and elevated urinary gonadotropin excretion titers. Initially, the etiologic basis for these findings was obscure. In 1956 the observations of Bradbury, Bunge, and Boccabella and those of Plunkett and Barr (1956) suggested that an X-chromosomal abnormality might explain the pathogenesis of Klinefelter's syndrome. These investigators detected sex chromatin bodies in the buccal mucosal cell nuclei of such patients. The basis for finding sex chromatin bodies in certain phenotypic males was clarified by Jacobs and Strong (1959), who demonstrated an XXY chromosomal pattern in a patient having Klinefelter's syndrome. Other X-chromosomal abnormalities have subse-

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[2] The format for the references cited in this article has been changed. References are divided into two parts. First, the 218 references that pertain to the literature review for clinical and laboratory features are listed. Second, all other cited references are listed in their usual fashion.

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3002420

quently been demonstrated in this condition. Furthermore, additional features have been noted to occur with sufficient frequency to be included in the syndrome.

The purpose of this report is to review certain clinical, chromosomal, histologic, and hormonal findings encountered in Klinefelter's syndrome. Particular attention will be paid to the importance of X-chromosomal mosaicism as a modifying influence in this disorder.

II. Clinical Features

The fundamental defect responsible for Klinefelter's syndrome is an overdosage of X-chromosomes. Therefore, any phenotypic male who demonstrates two or more X-chromosomes plus at least one Y-chromosome in all or in part of his body tissues should be included in this syndrome. Functionally, the presence or absence of supernumerary X-chromosomes in the testis determines whether or not seminiferous tubule and Leydig cell damage occurs.

Classically, the salient features of this disorder include (1) small, firm testes, (2) varying degrees of eunuchoidism, (3) azoospermia, (4) gynecomastia, (5) mental abnormalities, (6) chromatin positive buccal smear pattern, and (7) elevated urinary gonadotropin titers.

The clinical and laboratory features in these patients are present with varying frequencies. Table I is derived from a review of the English literature which covered the details of 1028 adults[6] with Klinefelter's syndrome in addition to 26 patients from our own laboratory (references 1-218).[7] Certain important features are depicted along with their percentage of frequency. Mental abnormalities were not tabulated because specific or objective data were lacking in too many instances.

Only those patients who demonstrated a chromatin-positive buccal smear pattern and/or evidence of an extra X-chromosome were included in our evaluation. Unfortunately this placed us in the position of excluding the patients originally described by Klinefelter *et al.* (1942) and those documented by Heller and Nelson (1945). Undoubtedly the majority of their patients were examples of the syndrome, but strict adherence to the afore-

[6] For purposes of classification only those patients who were at least 19 years of age were tabulated. If no age was stated in the article, the patient was included if the description was such that he could be considered an adult. This arbitrary approach was taken to avoid the problem of variable pubescence.

[7] Any case presented in two or more reports is included only once in the bibliography unless significantly new information was presented subsequently. Nonetheless, such patients are considered only once in the tabulation. This review extends through calendar year 1966.

3002421

mentioned criteria was considered necessary if we were to examine the literature in a consistent fashion.

The frequency with which certain features are described in any syndrome relates to the type of population being examined, the ability of the examiner to diagnose the syndrome, and his ability to detect and record the clinical and laboratory findings. The reported incidence of gynecomastia (50%) in Klinefelter's syndrome illustrates one of these points. In two large series of adults with this syndrome, the frequency of gynecomastia was 25 and 33% (Raboch, 1964; Court Brown *et al.*, 1964). This contrasts with earlier concepts (Klinefelter *et al.*, 1942; Heller and Nelson, 1945; Nelson and Heller, 1945; Sniffen *et al.*, 1951) and with our own experience, in which 23 of 26 patients (88%) had gynecomastia. The reason for this disparity is most likely due to the technique used for palpating the male breast. Many physicians consider that examining as for a mass in the female breast is adequate to detect the presence of gynecomastia. Pressing with the palm of the hand against the chest wall will enable the examiner to detect obvious instances of excessive breast tissue in the male, but less extreme examples of this abnormality will be missed. The correct procedure consists in compressing the subcutaneous tissue toward the nipple with the tips of the fingers. An area of increased consistency distinct from the surrounding soft tissue denotes gynecomastia. Nelson and Heller (1945) and Sniffen *et al.* (1951) have studied in detail the histologic characteristics of gynecomastia.

TABLE I

Clinical and Laboratory Features in Klinefelter's Syndrome

Pathologic feature	Frequency (%)	Patients: Number with disorder	Patients: Total examined
Histologic evidence of impaired spermatogenesis	99	292	294
Small testes	98	465	476
Azoospermia	95	194	206
Elevated urinary gonadotropin titer	83	210	253
Retarded facial hair growth	81	291	359
Impaired libido and/or sexual potentia	66	113	172
Gynecomastia	50	237	474
Retarded axillary hair growth	49	93	190
Retarded pubic hair growth	49	194	395
Impaired scrotal development	28	27	97
Impaired penile development	22	91	411

Defective spermatogenesis by histologic criteria is present in virtually all

3002422

patients (99%) with Klinefelter's syndrome. This results in small, firm testes (usually less than 2.0 cm, but always less than 3.5 cm in length) in 98% of patients. Azoospermia represents the next most common finding (95%). The frequency of these three features emphasizes the uniformity with which the fundamental defect, i.e., damage to the seminiferous tubules, appears in this syndrome.

Except for the gynecomastia, the remaining abnormalities listed in Table I are related to decreased Leydig cell function and/or the variable response by each end-organ system to given levels of testosterone. Thus the secondary sexual characteristics usually develop unequally. For example, only 22% of adults with Klinefelter's syndrome have a small penis whereas 81% exhibit decreased facial hair.

III. Chromosomal Studies

A. Buccal Smear Survey

Although the results of buccal smear surveys in newborn males indicate the incidence of Klinefelter's syndrome in the general population, it is difficult to correlate encountered X-chromosomal abnormalities with the eventual status

TABLE II

Results of a Buccal Smear Survey in Noninstitutionalized Adult Males[a]

Total number of slides examined	1097
Duplications	112
Chromatin-negative preparations not identifiable	8
Corrected total males examined	977
Chromatin-positive males	2
% Chromatin-positive males	0.2

[a] From Paulsen *et al.* (1964).

of the mature testis. Consequently, we conducted a survey of noninstitutionalized adult males who visited the outpatient department of a general hospital (Paulsen *et al.*, 1964). Along with our correlation we wished to test the following hypothesis: "Was it possible for a male with a buccal smear chromatin-positive pattern to exhibit normal testicular function?"

Table II lists the results of our survey. Of the two males with a "positive" buccal smear, one was a typical example of Klinefelter's syndrome. The other patient, P.S., with mosaicism, was of particular interest since he demonstrated very little pathology. P.S. was a 61-year-old single Caucasian merchant seaman. Physical examination, including the secondary sex characteristics, was normal, except for the presence of an atrophic right testis. His left testis was normal in size and consistency. According to the patient, his right testis was of normal size until he developed unilateral mumps orchitis at age

3002423

30. Gynecomastia was absent. Bilateral testicular biopsy specimens were obtained, but seminal fluid examination was declined by the patient. Tissue from skin, peripheral blood, and left testis were analyzed for their chromosomal complement. He proved to be an example of XY/XXY mosaicism. (See Tables III and IV for details of this patient's sex chromosomal pattern.)

B. Chromosome Studies

Since the results of our buccal smear survey indicated the importance of mosaicism in Klinefelter's syndrome, we searched for other examples. During the past four years we have had an opportunity to study five additional patients with varying degrees of decreased testicular function who demonstrated XY/XXY mosaicism. The methods employed for tissue culture and chromosome analysis were basically those of Moorhead *et al.* (1960) for peripheral blood; Harnden (1961) for skin; and Steinberger and Steinberger as described by Paulsen *et al.* (1964) for testis. Tables III and IV depict the data obtained from each of the six patients examined.

Skin culture preparations were not adequate for analysis in three patients (R.S., W.B., and F.H.) but the buccal smear pattern was compatible with a pure XY chromosomal complement, i.e., chromatin negative. In four patients, examination of the skin and/or buccal mucosa did not reflect the underlying X-chromosomal abnormality. More important was the fact that in three patients the overdosage of X-chromosomes was detected *only in the testis.* Therefore, in some instances, chromosome analysis of multiple tissues is essential to the diagnosis of Klinefelter's syndrome.

The data from patient F.H. illustrate another important point (see Table IV). That is, in the presence of mosaicism, one of the stem-cell lines may be represented by only a few metaphase plates and still be considered significant. Furthermore, with mosaicism the ratio between the stem-cell lines may not be the same when repeat observations are made at different points in time. In this regard, the experience of Hecht *et al.* (1966) is particularly important to note. These authors found that a second analysis was required before they obtained proper information. Their initial studies on an 18-year-old phenotypic male failed to demonstrate a Y-chromosome. Further culture preparations revealed a stem-cell line containing a Y-chromosome in 1% of their metaphase plates. They concluded that their patient was an example of XX/XXY mosaicism. The basis for this phenomenon in mosaicism has not been established. Some investigators have considered this to be an artifact of tissue culture. Gartler and Linder (1964) present evidence that suggests intercellular competition and selection occurring either *in vivo* or *in vitro* may be responsible for the variation seen in mosaic populations.

3002424

Testicular biopsy specimens from 21 normal adult males were submitted to chromosome analysis and used as controls for our patients with Klinefelter's syndrome. The results are given in Table V. Only 13 of 2368 cells contained

TABLE III

Sex Chromosome Complement in Patients with XY/XXY Mosaicism

Patient	Age	Skin	Blood	Testis
D.M.	19	XXY	XY/XXY	XY/XXY
P.S.	61	XY/XXY	XY/XXY[a]	XY/XXY
R.S.	23	—	XY/XXY	XY/XXY
R.L.S.	53	XY	XY	XY/XXY
W.B.	54	—	XY	XY/XXY
F.H.	49	—	XY	XY/XXY

[a] Originally (Paulsen *et al.*, 1964) we concluded that P.S. had a single stem-cell line (XY) in his peripheral blood preparation. Since then we have improved our technique for counting chromosomes, i.e., photographing all metaphase plates with a Zeiss photoscope. Chromosome counts are made from a projection of the negative strip film. Reexamination of his preparation revealed XY/XXY mosaicism.

TABLE IV

Analysis of Metaphase Plates from Testicular Tissue of Patients Exhibiting XY/XXY Mosaicism

	Chromosomal number			
	46		47	
Patient	Cells counted	Cells analyzed	Cells counted	Cells analyzed
D.M.	14	3	55	6
R.S.	244	26	6	6
F. H.	206	17	1	1[b]
R.L.S.	148	14	10	10
W.B.	108	17[a]	18	15[c]
P.S.	31	8	10	10

[a] One cell showed two X-chromosomes with a loss of an autosome.

[b, c] One cell with 48 chromosomes had an extra autosome plus two X-chromosomes.

TABLE V

Testicular Chromosomes in Normal Men

Number of subjects	Age	Number of cells	Number of chromosomes				
			< 45	45	46	47	> 47
21	22–49	2368	143	57	2256	11	2
			Cells analyzed[a]				
		433	7	54	360	11	1

[a] No XXY cells were noted.

3 0 0 2 4 2 5

more than 46 chromosomes. No extra X-chromosome could be detected in the 433 cells which were karyotyped.

C. Effect of XY/XXY Mosaicism on Clinical and Laboratory Features

From the material summarized in Table I, those patients who did not show evidence of mosaicism on chromosome analysis were evaluated separately. The features of these patients were then compared with the clinical and laboratory findings of patients whose skin, peripheral blood, and/or testicular preparations demonstrated XY/XXY mosaicism. The results of this comparison are depicted in Tables VI and VII. Although the sample size for each category is small, the data suggest that there is less pathology in patients with XY/XXY mosaicism. This is even more striking when the histologic characteristics of the testicular biopsy specimens are evaluated (see Section IV). The following case study illustrates the alteration in the clinical features which can be seen in some patients with XY/XXY mosaicism.

TABLE VI

Comparison of the Frequency of Certain Clinical Features in Patients with XXY and XY/XXY Mosaicism (Adults)[a]

Pathologic feature	Frequency (%)	Patients: Number with disorder	Patients: Total examined
Facial hair, decreased or absent			
XXY	72	77	107
XY/XXY	57	8	14
Pubic hair, decreased or absent			
XXY	61	76	125
XY/XXY	46	6	13
Axillary hair, decreased or absent			
XXY	46	50	108
XY/XXY	30	3	10
Length of penis, decreased			
XXY	41	50	121
XY/XXY	13	2	15
Gynecomastia, present			
XXY	45	53	118
XY/XXY	36	5	14

[a] By statistical analysis (chi square) $p = > 0.05$ in each category.

3002426

TABLE VII
Comparison of the Frequency of Testicular and Certain Hormonal Abnormalities in Patients with XXY and XY/XXY Mosaicism (Adults)[a]

Pathologic feature	Frequency (%)	Patients: Number with disorder	Patients: Total examined
Testicular histology, abnormal			
XXY	100	53	53
XY/XXY	91	10	11
Length of testis, decreased			
XXY	99	152	153
XY/XXY	65	13	20
Azoospermia			
XXY	92	22	24
XY/XXY	40	2	5
Plasma testosterone, decreased			
XXY	60	6	10
XY/XXY	40	2	5
Total urinary gonadotropins, increased			
XXY	75	38	51
XY/XXY	100	5	5

[a] By statistical analysis (chi square) $p = > 0.05$ except in the category length of testis $p = < 0.01$.

W.B., age 54 (see Tables III and IV), was admitted to the hospital complaining of tender gynecomastia, right breast, for 10 days. Further questioning revealed a history of his having been aware of bilaterally enlarged breast tissue since age 16. Puberty began at age 14-15 with no other apparent abnormality. At age 25 he developed mumps orchitis in the right testis. He claimed to have fathered two children prior to the episode of mumps and one child seven years after the orchitis. At age 42 he noted the onset of decreased libido and sexual potentia which led finally to complete loss of sexual interest. No change in secondary sexual characteristics had occurred. The only abnormalities observed on physical examination were the presence of bilateral gynecomastia and small, firm testes each measuring 2 cm in length (Fig. 1).

Routine laboratory findings were within normal limits. Endocrine studies revealed: (1) increased urinary FSH (Steelman-Pohley assay) titers of

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> 27.7 IU/24 hours, (2) decreased plasma testosterone of 0.18 μg%, and (3) elevated serum LH (radioimmunoassay) of 54.1 mIU/ml (see Section V for details of hormone studies).

It was not possible to study the genetics of the patient, wife, and children. Thus proof of his paternity could not be definitely established.

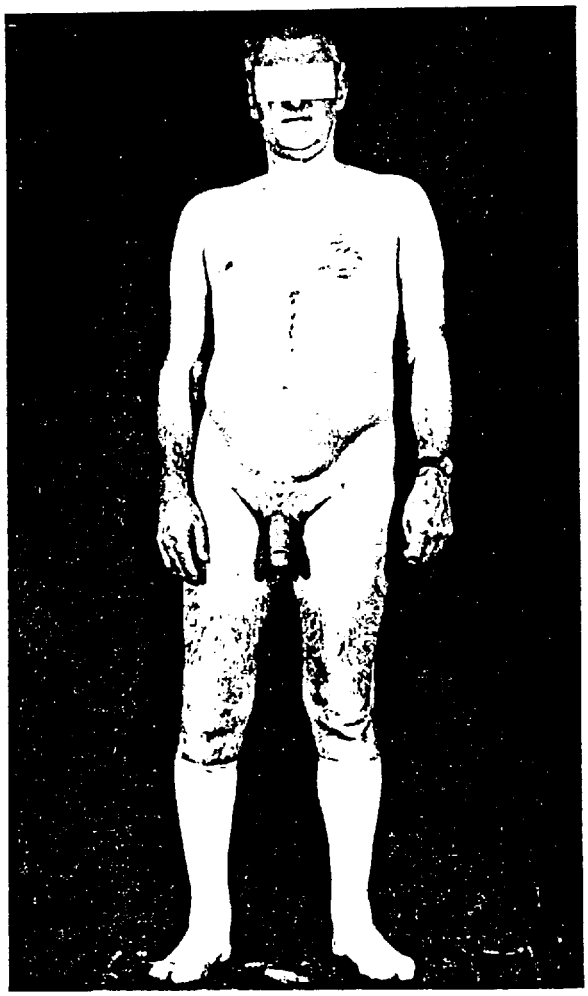

FIG. 1. Clinical photograph of patient W.B. The absent thigh hair is due to the surgical "prep" for his testicular biopsy.

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IV. Histologic Studies

Testicular biopsy specimens from patients with Klinefelter's syndrome usually reveal a uniform pattern of seminiferous tubular hyalinization and fibrosis with aspermatogenesis. Careful inspection of serial cross sections of the biopsy specimen or the entire testis may uncover small areas that contain less severely damaged tubules. Some contain just Sertoli cells; others may exhibit spermatogenesis (Bunge and Bradbury, 1956; Grumbach *et al.*, 1957; Witschi *et al.*, 1957; Ferguson-Smith and Munro, 1958; Steinberger *et al.*, 1965). Since testicular chromosome analyses were not performed in these instances the possible relationship between their findings and mosaicism could not be determined.

In order to evaluate the relationship between testicular morphology and XY/XXY mosaicism, testicular biopsy specimens were obtained from each of our six patients with XY/XXY mosaicism. A representative cross section from each specimen was selected for quantitation. All the seminiferous tubules were counted and tabulated according to their degree of pathology. Two additional patients with Klinefelter's syndrome who did not demonstrate testicular chromosome mosaicism, R.B. and R.C., were studied in a similar

TABLE VIII
Histologic Quantitation of Testicular Biopsy Specimens

Patient	Hyalinized	Sertoli	Spermatogonia	Spermatocytes	Spermatids
XXY testes					
R.B.	196	3	1	3	—
B.C.	63	10	—	—	—
XY/XXY testes					
D.M.	60	51	2	4	22
R.S.	26	—	63	37	21
F.H.	276	—	—	—	—
R.L.S.	50	—	3	9	96
W.B.	380	—	—	—	—
P.S.	67	—	5	2	54

fashion. The results are listed in Table VIII (also see Figs. 2-5). In four of the six patients with XY/XXY mosaicism, 14-61% of the seminiferous tubules contained mature or maturing spermatids as the most advanced germ cell type. Other tubules revealed spermatogonia or primary spermatocytes as the most advanced cell type, while 11-52% of the tubules were completely hyalinized and acellular. These findings are in contrast to the marked degree

3002429

of damage noted in those patients with Klinefelter's syndrome who are not examples of mosaicism (Ferguson-Smith, 1963).

The reports of fertility in patients with Klinefelter's syndrome offer further evidence as to the modifying influence of mosaicism. In each of the three males whose fertility has been established, XY/XXY mosaicism was present (Warburg, 1963; Dowling and Knox, 1963; Court Brown *et al.*, 1964). The

FIG. 2. Testicular biopsy specimen from patient P.S., an example of XY/XXY mosaicism. Spermatogenesis is present with maturation to mature spermatids. In this portion of the biopsy specimen the peritubular membranes are not fibrotic. × 175.

case report by Barr *et al.* (1962) is also consistent with this thesis. They investigated a mentally defective male who had XY/XXXY chromosomes in his skin, blood, and testis. Only minor alterations in spermatogenesis were detected on histologic examination of his testicular biopsy specimen.

Two of the six patients (R.S. and W.B., Table IX) had severe testicular damage at the time of investigation; therefore, the mere presence of an XY stem cell line in the testes of patients with Klinefelter's syndrome does not necessarily modify the degree of pathology. It may be that genetic phenomena such as penetrance or expression also enter into the picture.

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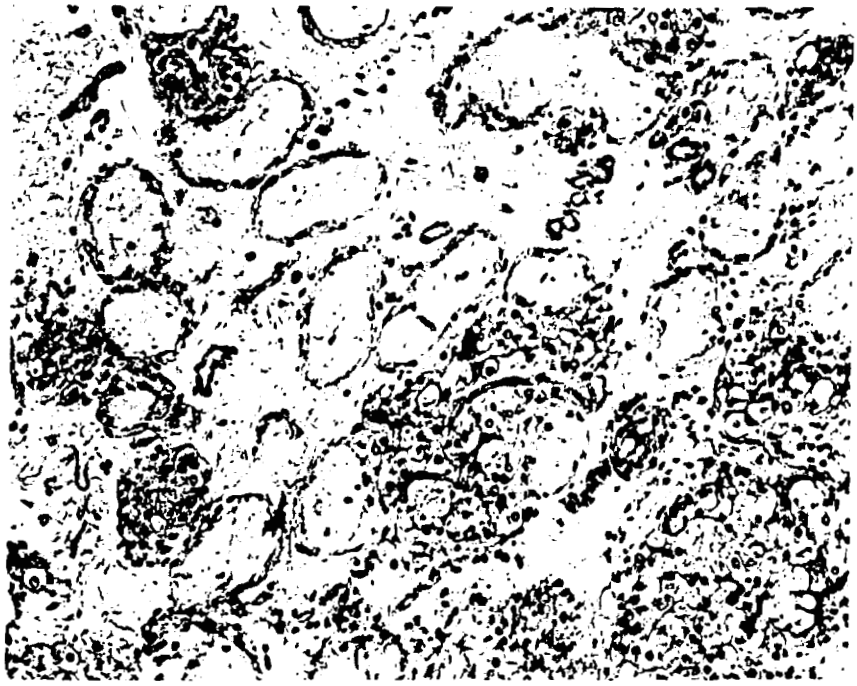

FIG. 3. Testicular biopsy specimen from patient W.B., another example of XY/XXY mosaicism. The seminiferous tubules are completely hyalinized and germinal cells are absent. Adenomatous clumping of Leydig cells can be seen in the interstitial spaces. The increased reticulum surrounding the hyalinized tubules suggests that damage occurred postpuberally. × 175.

FIG. 4. (a) Testicular biopsy specimen from patient R.L.S., XY/XXY mosaic. In this portion of the biopsy specimen there is moderate damage to the seminiferous tubules. Mature spermatids can be seen in two of the tubules. Leydig cells appear to be slightly increased in number. × 250. (b) Low power view, same patient. × 175.

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a

b

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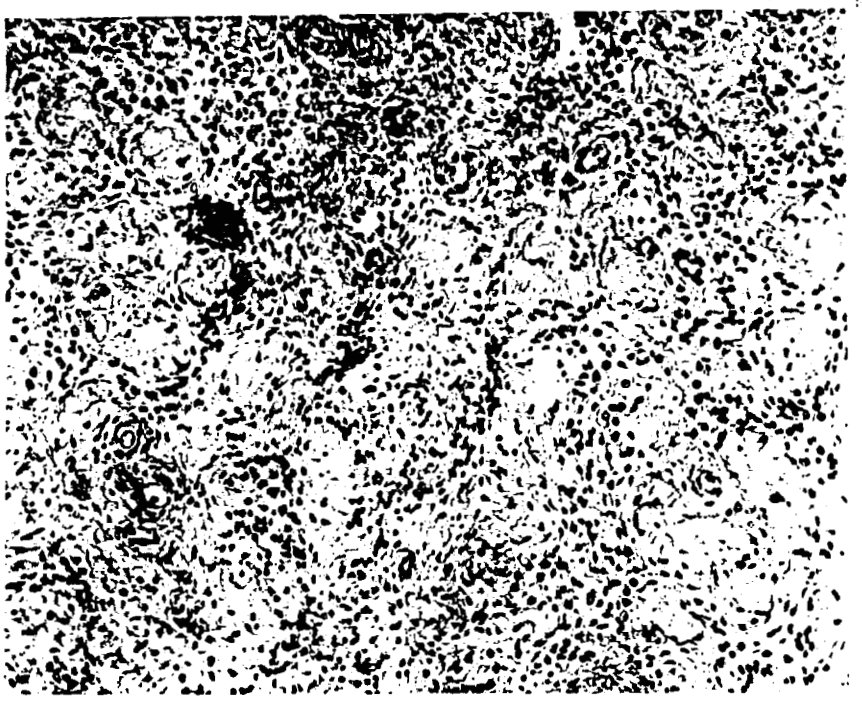

FIG. 5. Testicular biopsy specimen from patient R.B., "pure" XXY. The seminiferous tubules are completely hyalinized and devoid of germinal cells. In this patient Leydig cells are more fibroblastic in nature and distributed uniformly throughout the interstitial spaces. × 175.

V. Hormonal Studies

A. Plasma Androgens

Leydig cell function was evaluated in patients with Klinefelter's syndrome by measuring plasma testosterone concentrations. These results were then compared with values obtained from normal adult males. In addition, Δ^4-androstenedione concentrations were measured by a double isotope derivative technique utilizing ^{14}C indicator and ^{3}H acetic anhydride (Gandy and Peterson, 1968). The nonspecific blank of the method was 0.0017 ± 0.0002 (SE) and 0.0021 ± 0.0011 (SE) μg of steroid equivalent per 100 ml of water for testosterone and Δ^4-androstenedione, respectively. Listed values for these steroids have not been corrected for the blank. The coefficient of variation for concentrations in the normal adult male range were 2.3% for testosterone and 10% for Δ^4-androstenedione.

3002433

1. *Baseline Studies*

Plasma testosterone levels were determined in 25 patients with Klinefelter's syndrome (Table IX). Each blood specimen was obtained with a heparinized syringe between 0730 and 0900 hours. The values ranged between 0.03 and

TABLE IX
Plasma Testosterone and Δ^4-Androstenedione Levels in Patients with Klinefelter's Syndrome

Patient	Age	Buccal smear	Testosterone (μg%)	Δ^4-Androstenedione (μg%)
P.A.	26	+	0.23	0.09
W.B.	54	−	0.18	0.16
R.B.	19	+	0.14	0.06
J.B.	38	+	0.22	0.17
B.C.	36	+	0.32	0.31
P.C.	38	+	0.32	NT[a]
G.E.	21	+	0.45	NT
F.H.	49	−	0.05	0.02
Le.	22	+	0.23	NT
J.M.	41	+	0.07	0.04
D.M.	19	+	0.42	0.02
J.Mc.	23	+	0.21	NT
M.M.	38	+	0.03	NT
H.N.	66	+	0.42	0.14
R.N.	34	+	0.06	0.06
G.S.	22	+	0.15	0.23
W.S.	45	+	0.10	NT
P.S.	20	+	0.42	0.17
J.Sc.	35	+	0.08	0.07
J.S.	22	+	0.45	NT
R.L.S.	53	−	0.45	0.04
R.St.	39	+	0.88	NT
R.Su.	23	−	0.47	0.21
D.S.	19	+	0.31	NT
W.T.	29	+	0.45	0.07
Mean			0.28	0.12

[a] NT = not tested.

0.88 μg% with a mean of 0.28 μg%. For comparison, the morning baseline plasma testosterone concentration of 60 normal males, aged 20-45 years, varied from 0.28 to 1.44 μg% with a mean of 0.67 μg% (Fig. 6).

Briefer *et al.* (1965a) and Coppage and Cooner (1965) have previously reported that plasma testosterone levels in patients with chromatin-"positive"

3002434

Klinefelter's syndrome are invariably lower than the levels encountered in normal males. On the other hand, Hudson *et al.* (1963, 1965) and Lipsett *et al.* (1966) demonstrated overlapping between testosterone levels in normal males and patients with Klinefelter's syndrome. Our data also show that some patients with Klinefelter's syndrome have testosterone levels that are comparable to normal adult male levels.

Since Briefer *et al.* (1965a) reported that patients with so-called chromatin "negative" Klinefelter's syndrome have normal plasma testosterone levels,

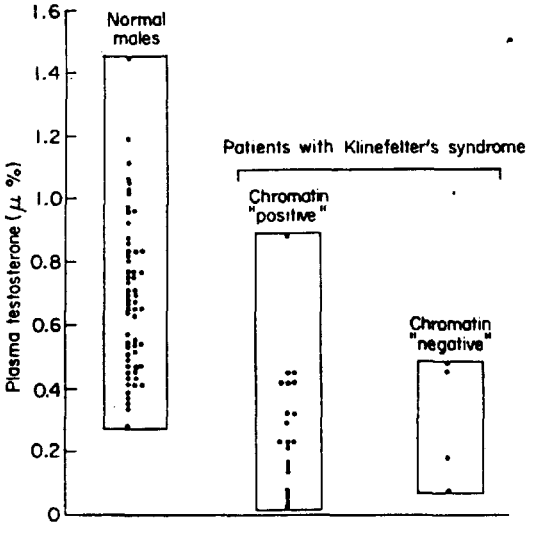

FIG. 6. Plasma testosterone baseline levels in normal adult males and patients with Klinefelter's syndrome. The patients have been divided into two groups according to their buccal smear chromatin pattern.

we separated our patients according to their buccal smear pattern (Fig. 6). In the chromatin "negative" category, two patients had testosterone concentrations in the normal range (0.45 and 0.47 μg%) while two others had values that were below normal (0.05 and 0.18 μg%).

Our testosterone data plus the detailed chromosomal analyses mentioned previously in this report demonstrate that a classification of Klinefelter's syndrome based on the chromatin pattern of the skin represents an *improper* line of division.

Due to the inherent difficulty in correcting for the duration of postpuberal Leydig cell function and any regression thereof, no effort was made to correlate testosterone titers with the degree of secondary sexual development.

3002435

However, from a clinical viewpoint only one patient was considered to be normally virilized *and* to exhibit normal libido and sexual potentia at the time of our examination (R.St., Table IX).

Plasma Δ^4-androstenedione levels were measured in 16 patients with Klinefelter's syndrome. Values ranged between 0.02 and 0.31 μg% with a mean of 0.12 μg%. This compares with titers of 0.06-0.23 μg% found in 41 normal men (Gandy and Peterson, 1968). These data suggest that there is no significant increase of the testosterone precursor, androstenedione, by the Leydig cells of patients with Klinefelter's syndrome.

2. *Response to Human Chorionic Gonadotropin (HCG) Administration*

Hudson *et al.* (1965), Briefer *et al.* (1965b), and Lipsett *et al.* (1965) have concluded that plasma testosterone levels do not significantly increase when HCG is administered to patients with Klinefelter's syndrome. Thus the Leydig cells of these patients may already be maximally stimulated by endogenous gonadotropin. These observations, however, are at variance with the observations of Leach *et al.* (1956), Leon *et al.* (1959), and Paulsen (1965), who demonstrated either increased sexual development, increased 17-ketosteroid excretion, increased urinary estrogen excretion or mitotic activity of Leydig cells after long-term HCG administration. In order to study this problem further, 5000 IU of HCG was administered to 8 normal adult males and 10 patients with Klinefelter's syndrome as a "1-day test."

Plasma testosterone was measured prior to and 8 hours after HCG administration in each subject (Fig. 7). A distinct rise in plasma testosterone concentration occurred in three of the patients with Klinefelter's syndrome (increments of 0.22, 0.34, and 0.37 μg%). No change or a decrease in testosterone levels was observed in the remaining eight patients. In the normal males, testosterone concentration increased significantly in four, remained essentially the same in two, and decreased in two.

Since Leydig cell stimulation was not uniform in the normal males, the study was repeated and the duration of HCG administration was extended to 4 days. Blood samples were obtained prior to HCG administration and at 1600 hours on each of the 4 days (Fig. 8). Plasma testosterone levels steadily increased throughout the 4 days in all but one of the normal men. Testosterone values on the fourth day were from 0.20 to 1.01 μg% (mean 0.71 μg%) higher than control values. Six of the eight patients with Klinefelter's syndrome responded to the HCG administration. In these patients plasma testosterone levels reached a peak on day 3 and declined on day 4. The testosterone levels on day 3 were higher than control levels by 0.26-0.73 μg% (mean 0.41 μg%).

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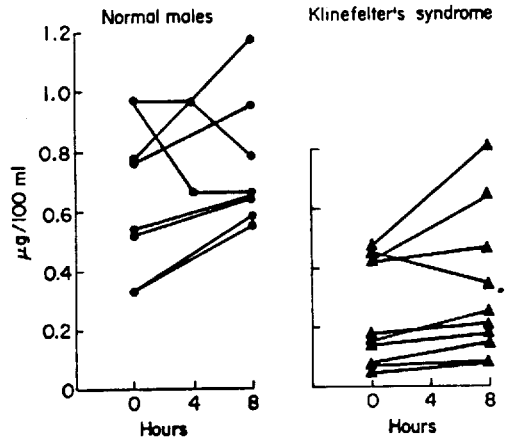

FIG. 7. Plasma testosterone levels in normal males and patients with Klinefelter's syndrome before and after administration of human chorionic gonadotropin (HCG). HCG was injected intramuscularly between 0800 and 0830 hours after the control plasma sample was obtained.

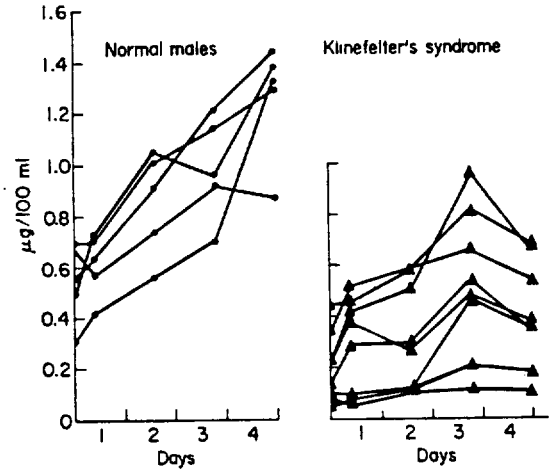

FIG. 8. Plasma testosterone levels in normal males and patients with Klinefelter's syndrome during 4 days of human chorionic gonadotropin administration. HCG was administered each morning for 4 days (5000 IU at 0800 hours).

3002437

These data demonstrate that the Leydig cells in patients with Klinefelter's syndrome can respond to HCG stimulation but have a limited reserve.

B. Urinary Gonadotropin Excretion

The urinary gonadotropin excretion titers of our patients with Klinefelter's syndrome were studied by two methods (Table X). Total or general gonadotropins (GGA) were measured by the method of Albert (1956). This technique determines the combined biologic effects of FSH and ICSH or LH. In seventeen patients the GGA titers varied from 2.8 to 17.6 mg-eq of UPM-1

TABLE X

Urinary Gonadotropin Excretion Titers in Patients with Klinefelter's Syndrome

Patient	Age	GGA[a] (mg-eq UPM-1/24 hr)	FSH[b] (IU/24 hr)
W.B.	54	—	74.5
R.B.	19	—	73.1
R.Bau.	28	3.7	—
H.B.	62	2.8	—
B.C.	36	6.6	—
P.C.	38	11.1	132.5
K.C.	48	7.7	56.0
G.E.	21	—	131.7
F.H.	49	13.4	204.6
W.H.	45	16.0	69.0
D.L.	19	17.6	—
J.M.	41	—	140.0
D.M.	19	5.1	58.0
J.Mc.	23	—	71.2
M.M.	38	6.3	—
W.N.	22	16.2	195.8
G.S.	22	13.6	76.7
W.S.	45	5.6	87.6
J.S.	22	—	63.9
R.L.S.	53	9.5	124.2
R.St.	39	10.0	—
R.Su.	23	4.9	78.5
D.S.	19	8.8	—

[a] Total or general gonadotropin assays performed by method of Albert (1956). Results are expressed as milligram-equivalents of the NIH standard, UPM-1. Normal range for adult males: 0.7-3.8 mg-eq. per 24 hours. The computer program of Thorslund and Paulsen (1963) was used for statistical analysis.

[b] FSH assays performed by the method of Steelman and Pohley (1953). In our assay 50 IU of HCG was used as the "priming" dose. Results are expressed in terms of the FSH potency established for the 2nd I.R.P. Normal range for adult males: 3.8-20.0 IU per 24 hours.

3002438

per 24 hours (range for normal adult males 0.7-3.8 mg-eq). The single patient with a normal GGA titer (2.8 mg-eq) was a typical example of Klinefelter's syndrome. Clinically, his Leydig cell function was considered to be normal, but plasma testosterone was not measured. He was of average intelligence and there was no evidence of either central nervous system disease or a generalized catabolic disorder. His testicular biopsy specimen revealed complete hyalinization of the seminiferous tubules.

Specific FSH titers were measured in sixteen patients by the Steelman-Pohley (1953) method. Elevated titers were noted in each patient. These varied from 56.0 to 204.6 IU per 24 hours (range for normal adult males 3.8-25.0 IU per 24 hours).

Although the details are not clearly understood with respect to the interrelationship between FSH secretion and germinal cell activity (Paulsen, 1968), it stands that in the presence of significant primary seminiferous tubular disease, such as occurs in Klinefelter's syndrome, FSH levels are elevated. Thus it was not surprising to find uniformly elevated FSH levels in our patients.

There are considerable data to support the existence of a negative feedback mechanism for the regulation of LH and testosterone levels (Lipsett and Korenman, 1964; Kirshner *et al.*, 1965; Lipsett *et al.*, 1966). Urinary LH assays were performed only rarely in our patients, therefore we have not tabulated our results. Becker and Albert (1965) documented increased urinary FSH and LH levels in one patient with Klinefelter's syndrome. Information is lacking, though, with regard to simultaneous comparisons between LH levels and plasma testosterone concentration. To examine this issue, we turned to the sensitive radioimmunoassay technique for measuring serum LH levels.

C. Radioimmunoassay Studies

Serum human luteinizing hormone (HLH) was measured by the double antibody radioimmunoassay technique (Morgan *et al.*, 1964) as adapted for human chorionic gonadotropin (HCG) and HLH (Midgley, 1966). Anti-HCG antiserum was prepared and absorbed according to the method of Midgley. Specificity of the absorbed antiserum to purified HCG was established by immunoelectrophoresis.

Each serum sample was assayed in duplicate; most samples were assayed three or more separate times. The average interassay variation for the individual means was 10.7%. During our studies a normal male serum pool was measured in each assay for internal control. The mean HLH value for this

3002439

pool in terms of the second international reference preparation was 14.3 mIU per ml ± 1.2 (SD).

In order to establish baseline values for normal adult males in our laboratory, the sera from 72 men were assayed for HLH titers. Thirty-four of the men were proved to be normal by physical examination, seminal fluid studies, and/or plasma testosterone determination. The remaining 38 men were judged to be normal by history and general appearance. Since the results for each group were similar, their serum HLH titers were combined (Fig. 9). The mean serum HLH was 10.8 mIU/ml (range 4-19 mIU). These data

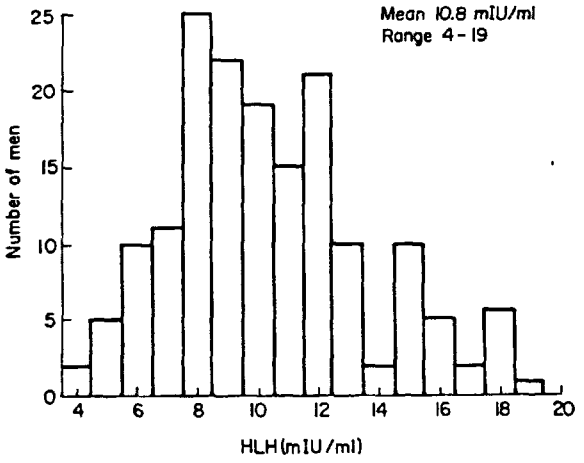

FIG. 9. Serum HLH levels in 72 normal adult males. In some men multiple serum specimens were assayed.

compare favorably with the values obtained by Odell *et al.* (1967). Their normal range varied between 4.8 and 25.6 mIU/ml.

Serum HLH titers were measured in twenty patients with Klinefelter's syndrome. Elevated levels ranging from 21.2 to > 128 mIU/ml were present in all but one patient (Table XI). Comparison of these values with plasma testosterone levels obtained from the same blood sample revealed that serum HLH titers were increased in 10 patients despite evidence of normal testosterone levels.

Although Midgley (1966) and Odell *et al.* (1967) demonstrated that no cross-reaction existed between FSH and HLH by immunoassay, it is possible that the high FSH titers present in our patients with Klinefelter's syndrome influenced our assay of their serum HLH titers. Therefore, additional studies of our assay technique were performed. First, a patient with Sertoli-cell-only

3002440

syndrome was evaluated. Patients with this defect exhibit elevated FSH levels but have normal urinary LH excretion titers since their testicular lesion is confined to the seminiferous tubules. The serum HLH level of the patient

TABLE XI

Comparison between Serum HLH and Plasma Testosterone Levels in Patients with Klinefelter's Syndrome

Patient[b]	Serum HLH[a] (mIU/ml) Range[c]	Mean	Plasma testosterone[a] (μg%)
P.A.	(13.2– 22.6)	16.5	0.23
W.B.	(53.0– 56.0)	54.1	0.18
W.B.	(25.5– 50.5)	42.9	0.14
R.B.	(33.5– 61.0)	48.2	0.14
J.B.	(28.0– 34.0)	30.9	0.04
B.C.	(54.0– 80.0)	67.0	0.32
P.F.C.	(> 128)	> 128.0	0.32
G.E.	(30.0– 61.0)	53.0	0.41
G.E.	(34.8– 38.1)	36.0	0.45
F.H.	(34.5– 45.2)	41.0	0.08
J.M.	(31.0– 37.2)	34.2	0.05
D.M.	(24.0– 32.0)	28.7	0.42
J.Mc.	(52.9– 54.0)	53.5	0.21
H.N.	(32.0– 44.5)	39.4	0.42
G.S.	(42.0– 86.0)	65.0	0.15
W.S.	(28.1– 44.0)	36.0	0.24
W.S.	(> 128)	> 128.0	0.10
W.S.	(61.5– 64.0)	62.7	0.12
J.Sc.	(24.0– 33.8)	29.1	0.08
J.Se.	(31.0– 52.9)	49.2	0.23
J.Se.	(29.5– 38.2)	35.1	0.45
R.St.	(31.0– 40.5)	34.0	0.88
R.Su.	(18.0– 23.5)	21.2	0.47
R.Su.	(34.0– 44.0)	39.0	0.35
D.S.	(70.0–100.6)	90.2	0.37
W.T.	(22.6– 24.0)	23.3	0.45

[a] Serum and plasma were obtained from the patient at the same time.
[b] See Table IX for ages of patients listed here.
[c] Each serum sample was assayed for its HLH titer in two to five separate assays.

evaluated was in the normal male range (Fig. 10), which offers additional evidence that elevated FSH levels do not interfere with the measurement of HLH by radioimmunoassay. Also the serum of a hypophysectomized male revealed undetectable levels of HLH. This indicates that we are not detecting nonspecific factors in our assay procedure.

3002441

Finally, a purified human pituitary gonadotropin preparation (LER 780[8]) with an LH bioassay potency of 430 IU/mg was compared to the second international reference preparation (2nd IRP) (Fig. 11). LER 780 by bio-

TABLE XII

Comparison between Serum HLH and Plasma Testosterone Levels in Normal Men

Subject	Serum HLH[a] (mIU/ml) Range[b]	Mean	Plasma testosterone[a] (μg%)
M.B.	(7.2– 7.8)	7.5	0.45
W.B.	(8.3– 8.4)	8.4	0.94
W.B.	(8.6– 9.2)	8.9	1.20
W.B.	(7.4– 7.7)	7.6	0.91
W.B.	(10.0–12.5)	11.3	1.13
L.B.	(10.0–10.2)	10.1	0.59
R.C.	(7.7–10.5)	9.1	0.51
L.C.	(6.6–10.5)	8.0	0.70
E.E.	(7.3– 9.5)	8.4	0.97
T.F.	(7.5– 9.4)	8.5	0.55
G.H.	(5.5– 6.0)	5.8	0.31
E.J.	(9.2–12.2)	10.7	0.55
E.M.	(9.2–10.8)	10.0	0.50
R.N.	(6.0– 7.2)	6.6	0.31
R.N.	(7.2–19.0)	13.1	0.32
R.N.	(8.8– 9.2)	9.0	0.36
R.N.	(7.7– 8.0)	7.9	0.42
C.P.	(8.4–10.7)	9.6	0.56
W.S.	(6.5– 7.8)	7.2	0.52
W.S.	(11.4–14.5)	13.0	0.75
W.S.	(11.7–12.5)	12.1	0.79
W.S.	(14.8–16.2)	15.5	0.67
A.S.	(10.0–13.7)	15.5	0.32
R.S.	(15.0–16.3)	15.7	0.67
R.S.	(12.3–21.0)	16.8	0.57
S.S.	(10.4–14.7)	12.6	0.64
J.Z.	(11.0–14.7)	12.9	0.51
J.Z.	(12.5–14.2)	13.4	0.79
J.Z.	(11.5–14.2)	12.9	0.59
J.Z.	(10.7–12.5)	11.6	0.75

[a] Serum and plasma were obtained from the patient at same time.
[b] Each serum sample was assayed for its HLH titer in two to five separate assays.

assay has an FSH/LH ratio of 3:1 whereas by definition the FSH/LH ratio of the 2nd IRP is 1:1. The dose response curves are comparable, again sug-

[8] Prepared by Leo E. Reichert, Jr. and supplied by the National Pituitary Agency.

3002442

gesting that alterations in FSH/LH ratios do not influence the determination of HLH by radioimmunoassay.

Another point that deserves attention concerns the interpretation of plasma testosterone levels. Does the presence of a normal plasma testosterone concentration indicate normal testosterone production? Hudson *et al.* (1964, 1967) examined this problem, and their observations suggest that no major

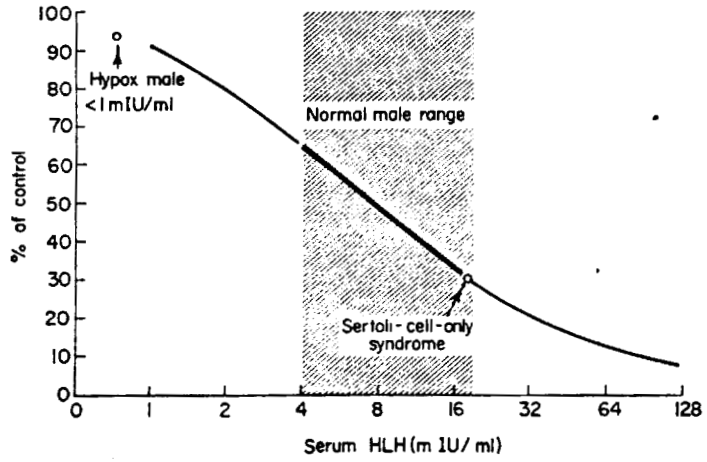

FIG. 10. Serum HLH assay results in a male with Sertoli-cell-only syndrome and a male after hypophysectomy. The shaded area indicates our normal male range.

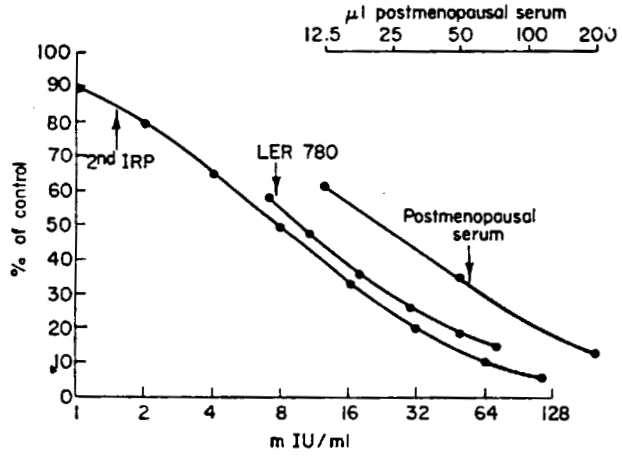

FIG. 11. The LH potency of the second international reference preparation and LER 780 by radioimmunoassay. FSH/LH ratio of 2nd IRP = 1:1; LER 780 = 3:1.

3002443

discrepancy exists in males between plasma levels and production rate. Normal males and five patients with Klinefelter's syndrome were included in their investigation. However, patients with Klinefelter's syndrome who exhibit *normal* testosterone levels associated with *diminished* secondary sexual characteristics have not been investigated thoroughly. It is possible that in our ten patients testosterone production was significantly reduced while plasma testosterone levels were only affected to a minor degree. If so, the serum HLH levels would serve as a more accurate and sensitive indicator of Leydig cell function than plasma testosterone levels. More study will be required to resolve this issue.

VI. Conclusions

1. The clinical features of Klinefelter's syndrome may be less severe when sex chromosomal mosaicism exists.
2. The presence of an XXY stem-cell line in the testis is associated with Klinefelter's syndrome even if it cannot be detected in the skin and/or peripheral blood.
3. Despite the presence of normal plasma testosterone titers in some patients, the elevated serum HLH levels suggests that Leydig cell function is abnormal.

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3002444

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3002451

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Discussion

C. G. Heller: The slides of testicular biopsy specimens that Dr. Paulsen showed us revealed two different situations as far as Leydig cells are concerned. One group revealed clumping of the Leydig cells in large masses, which suggests an increase in the total numbers of Leydig cells. The other group revealed a diffuse distribution of the Leydig cells—again, however, suggesting an increase in numbers. Joyce Pearson, in our laboratory is undertaking a study in which Leydig cells are being quantitated. For Klinefelter's syndrome, relating total numbers of cells to total numbers of seminiferous tubules (the tubules being considered as the constant) we find two situations. One in which Leydig cells are indeed not increased in total numbers occurs where the cells are distributed evenly. The apparent increase is accounted for by the decrease in diameter and length of the tubules. In the second situation, in which a great deal of clumping is encountered, there is indeed a very great increase in total numbers of Leydig cells versus the normal testicular biopsy. Thus in Klinefelter's syndrome one finds instances of normal Leydig cell numbers as well as instances of increased Leydig cell numbers. These findings might well correlate with the amount of testosterone produced by the testes in Klinefelter's syndrome as demonstrated by Dr. Paulsen.

C. A. Paulsen: The studies which you mentioned are quite interesting. J. W. Sargent

312452

and J. R. McDonald [*Proc. Mayo Clinic* **23**, 249 (1948)] proposed a similar method for the quantitative estimate of Leydig cells in the human testis. Preliminary studies were carried out in normal males and patients with testicular disorders, including Klinefelter's syndrome. These authors concluded that the number of Leydig cells was not increased in patients with Klinefelter's syndrome [J. W. Sargent, Master's Thesis, Mayo Clinic (1949)]. Since their study of Klinefelter's syndrome was somewhat limited, I am glad to see further work along this line.

[*Addendum note:* Patient W. B. (see Fig. 3) exhibited adenomatous clumps of Leydig cells; his plasma testosterone titer was 0.18 μg %. Patient R. B. (see Fig. 5) exhibited diffusely distributed Leydig cells; his plasma testosterone titer was 0.14 μg %.]

C. G. Heller: Only yesterday I had a discussion with Dr. Lloyd about one point that you made. It's a point that I never really thought about before. That is the comment that, no matter how much you treat people having this syndrome, some are never brought to normal. I find that this applies to Klinefelter's syndrome, and also to each of the syndromes of hypogonadism we encounter. Some individuals, no matter how much or how long you treat them, do not achieve normal facial hair growth. Other secondary sex characteristics of these individuals respond rather rapidly and do mature normally. You may wonder why facial hair should be so important. Not just facial hair but also facial expression is important to the individual, and I related to Dr. Lloyd the case of a young attorney from Vancouver, B. C., who was satisfied with the physical response to therapy, as was I, until he joined the Canadian diplomatic corps. When he had to deal with other diplomats, he thought he was at a tremendous disadvantage to have a baby face and not to be shaving. As his career developed and he was dealing with higher and higher authorities he came back more frequently for additional treatment, but we never were able to achieve complete normalcy.

Dr. Paulsen mentioned his tissue culture but he did not mention exactly what cells he is counting. I would like additional information.

As to the onset of Klinefelter's syndrome, we can confirm that the testes can indeed be normal as can urinary gonadotropins at about the time of puberty. We have one example of a youngster that we followed from before puberty, through puberty until he developed Klinefelter's syndrome at about 17-18 years of age. At this time he had the entire syndrome. His gonadotropins before puberty were absent, after puberty they were normal, and after he developed Klinefelter's syndrome they were elevated. I was very glad to see the revision of Dr. Paulsen's human chorionic gonadotropin test. He again showed the 8-hour test which was quite unsatisfactory. We have been very critical of this 8-hour test because in our own laboratory we find that the first significant rise in urinary testosterone and/or estrogen excretion occurs at 4 days, and the peak is reached in 7 or 8 days. Apparently Dr. Paulsen became aware of this himself, having developed a 4-day test which I accept as being totally satisfactory.

C. A. Paulsen: Briefly, the method for testicular tissue culture is as follows: (1) A portion of the biopsy specimen is minced and trypsinized. (2) The cells are then placed into TC medium 199 and 20% fetal calf serum. (3) After centrifugation, the cells are rinsed in Hank's solution and resuspended in the culture media. (4) Incubation is carried out at 37°C for approximately 7-10 days. (5) Initially the culture solution contains 500,000-700,000 cells per milliliter of media.

Identification of the cells which multiply under these conditions has not been fully established. Virtually all cells revert to a fibroblastic form when cultured in monolayer, thus morphologic characterization is not possible. Available evidence indicates that germ

3002453

cells do not survive. E. Steinberger, O. Vilar, and A. Steinberger [*Program 47th Meeting Endocrine Soc.*, p. 58 (1965)] demonstrated that rat testicular cells in monolayer give a positive cytochemical reaction for 3β-ol-dehydrogenase. This result suggests that we are analyzing the chromosomal configuration of Leydig cells. It is possible that other cellular elements from the interstitial spaces are also involved.

In regard to when the pathologic features develop in Klinefelter's syndrome, I would agree that this time can be variable. In most patients pathology is evident at puberty or very shortly thereafter. In some patients damage is not manifest until later in life. From our limited experience, a delay in development of testicular dysfunction is not necessarily confined to those patients who exhibit XY/XXY mosaicism. For example, patient B.C. in our series (single stem-cell line XXY) stated that his testes were of normal size until circa age 28. Furthermore he claimed to have produced two children. Unfortunately we were unable to study the genetics of his family to substantiate his statement. But we did not have any reason to doubt his history. We visualize that many factors influence the rate and degree with which pathology develops in Klinefelter's syndrome and liken this to the situation that exists in diabetes mellitus with its variable pattern.

C. W. Lloyd: I would like to extend the discussion Dr. Heller and I had yesterday. This is apropos of your comments about the possibility that the testosterone levels within the normal range may not be normal for these individuals. I think this is possible, but it seems to be a risky hypothesis unless there is other evidence. Have you any data to support this? One simple experiment would seem to me to be to show that if you raise the testosterone level by exogenous therapy you would bring the LH down. You did not mention this. If this were on the basis that the hypothalamus has a different setting, then raising the testosterone very high ought to bring the LH down. In other words, this would be analogous to the Cushing ACTH situation. As Dr. Heller said, these men don't really get complete replacement when you give them testosterone in very large doses. They still don't grow normal beards. It makes me wonder whether we might not have a situation in the human analogous to what you have in the rat where the mother is treated with an antiandrogen and you prevent masculinization of the fetus. There is evidence that this animal is not normally sensitive to androgens as is an adult. Perhaps in this particular strain of human we have an experiment in which in early fetal life they did not get the usual squirt of androgen, whenever it comes, and therefore they did not develop normal sensitivity of androgen-responsive tissues.

C. A. Paulsen: With regard to your second comment, I have no further information to add. In regard to your first point, we have measured total urinary gonadotropin excretion titers in some of these patients before, during, and after parenteral testosterone administration. Testosterone inhibited gonadotropin excretion in normal males and patients with Klinefelter's syndrome at comparable dose levels. This indicates that the pituitary-hypothalamic system is responsive to steroid suppression in patients with Klinefelter's syndrome. However, testosterone infusion studies need to be performed. With simultaneous measurement of serum HLH and testosterone titers it should be possible to determine the exact testosterone level required to "shut off" HLH secretion.

M. M. Grumbach: An interesting bit of evidence we have at present is the discrepancy between the circulating level of LH and of testosterone in these patients. If the hypothalamic regulatory center for LH release is not abnormally resistant to testosterone, then impaired Leydig cell function must be considered as a possible explanation of these observations.

3002454

G. S. Gordan: Dr. Paulsen has been extremely cautious and has not been speculating. In his presentation, Dr. Wool told us that there are certain fields where a trivial investment of fact may yield a bountiful harvest in theory. This seems to be the reverse. Undoubtedly Dr. Paulsen has some idea of why the LH is high in the presence of what appears to be a normal testosterone level. I wonder if in this particular method for measuring testosterone the inactive epi-isomer might be included. Would you tell us your ideas on why LH is high while testosterone is normal. My second question relates to what seemed to me a rather astonishing remark. When Klinefelter, Reifenstein, and Albright first described this syndrome it included gynecomastia. Then when Murray Barr came out with a method for sexing cells, the clinical phenomena changed entirely and gynecomastia virtually disappeared. You remarked that this is because we don't examine properly for gynecomastia; I must admit I don't know this trick and I would be grateful if you tell us how to do it.

C. A. Paulsen: Epitestosterone is removed by chromatography and does not interfere with the determination. Perhaps Dr. Gandy would amplify this.

H. M. Gandy: I have little to add. The one compound (epitestosterone) that I think might interfere with the measurement of testosterone is separated from testosterone by paper chromatography prior to making the acetate derivative of testosterone.

J. F. Tait: May I congratulate Dr. Gandy. I think only those who have developed labeled reagent methods can realize the amount of work involved in this study.

I would like to ask whether you measured also blood and urinary production rates. Due to the work mainly of Pearlman on testosterone binding to plasma proteins, by analogy with other endocrine fields one might expect to get better correlation with biological activity and perhaps also with plasma LH levels with the unbound concentration in plasma. This is rather difficult to measure accurately simply because it is difficult to extrapolate diluted plasma measurements to the undiluted plasma. Recently, I have concluded (*Perspectives Med. Biol.*) that the unbound concentration is probably in the case of testosterone proportional to the total blood production rates for subjects who have normal hepatic function. It is interesting in this regard that Bardin and Lipsett in hirsute women have gotten better correlation of clinical signs and symptoms with the blood production rate than with the total testosterone concentration. This is rather mysterious until one realizes that the blood production rate may be correlated with the unbound concentration. In males the urinary production rates may be correlated with the blood production rates and here with the unbound plasma concentration so that although I'd hate to lead a movement back to urine I also ask you whether you measured the urinary production rates or even the urinary excretion of testosterone glucuronide.

C. A. Paulsen: We did not study blood or urinary production rates, nor did we measure urinary testosterone glucuronide excretion. When Bryan Hudson studied testosterone blood production rates in his patients with Klinefelter's syndrome he did not uncover any significant difference between production rate and plasma testosterone concentration. However, in a few patients with low plasma testosterone levels the urinary production rates were higher. He concluded that this was due to the conversion of Δ^4-androstenedione to testosterone by the liver. Lipsett has emphasized this same problem in evaluating testosterone levels in women.

G. T. Bryan: With regard to your normal male population, was there any correlation between the age of these individuals and their plasma testosterone levels?

C. A. Paulsen: In our normal males, age 20-45 years, plasma levels did not decline with age. From preliminary data in men over 45 years of age there does not appear

3002455

to be any striking decline in plasma testosterone titers. In contrast, in patients with Klinefelter's syndrome testosterone levels may decline with age (Fig. A).

W. McK. Jefferies: Beard growth is not always a suitable criterion of androgen effect because of racial variation. For example, an American Indian does not grow a beard with normal androgen levels. Have you in your clinical observations noted any other evidence of resistance to androgen therapy in these patients?

C. A. Paulsen: As you indicated, ethnic factors will influence facial hair growth. However, this problem can be eliminated by careful history taking. We would agree with Dr. Heller that in some patients with Klinefelter's syndrome androgen therapy does not produce an "adult facies." Also it has been our impression that proper muscle development may be resistant to androgen therapy. Obviously, objective evaluation of these parameters is difficult.

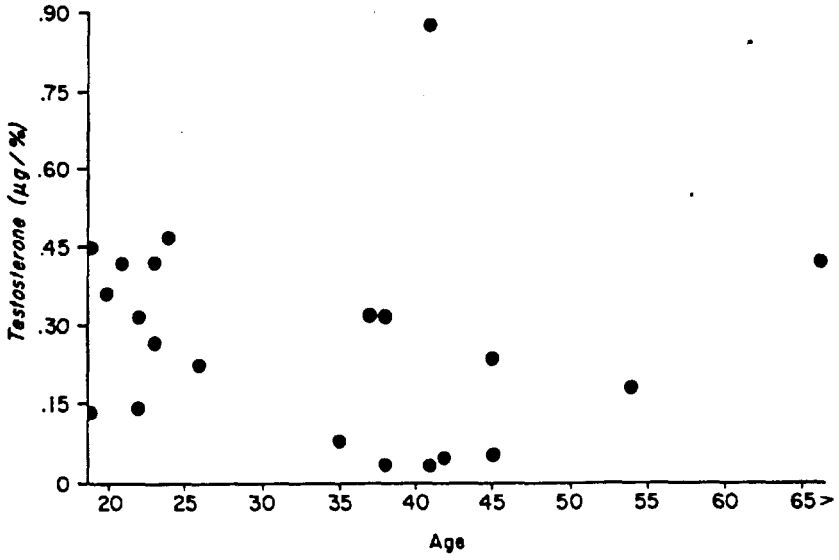

FIG. A. Plasma testosterone level versus age in Klinefelter's syndrome.

G. T. Ross: There is one other possibility that should be mentioned with respect to speculations about the disparity between plasma testosterone levels and plasma immunoreactive LH levels. Perhaps these patients might make bad LH, possibly on a genetic basis, since the pituitary may have bad chromosomes as well. This concept of an abnormality in LH is highly speculative, but not altogether without some basis in experience with respect to the radioimmunoassay procedure. I think it was unfortunate that it was not possible for you to have good biological estimates of the urinary excretion of LH activity in these patients to see whether or not these levels which you measured in plasma were related to urinary excretion, as one would expect them to be in normals. This is admittedly a variety of circumstantial evidence, but until we have better methods for estimating biological activity that do not require prohibitively large volumes of plasma, we may have to be content with this type of indirect evidence.

3002456

C. A. Paulsen: I agree that a dichotomy between immunologically reactive HLH and biologically active HLH may exist. This has been demonstrated for ACTH in certain patients but so far not for pituitary gonadotropins. Your suggestion regarding the presence of a "bad" HLH certainly deserves study. We did not emphasize our bioassay results with respect to urinary LH since we had valid assays in only four patients. In two, the urinary LH excretion titers were in the normal male range, and in two patients the LH values were elevated. Serum HLH was increased in all four, range 34.2-65.0 mIU/ml. Plasma testosterone levels were low in each patient, ranging from 0.07 to 0.18 μg %. Thus, we lack LH bioassay data in patients who demonstrate normal plasma testosterone levels and elevated serum HLH. Other investigators have found elevated LH levels (bioassay) in patients with Klinefelter's syndrome, but no correlation has been made with serum HLH or plasma testosterone levels.

R. A. Huseby: Regarding the "inappropriate" levels of LH and testosterone, there seems to be yet another alternative explanation. This is that the interstitial cells may be relatively insensitive to LH. Certainly this is the situation we encounter in the surgically cryptorchid mouse. The Leydig cells appear normal histologically, and the production of androgen actually is increased. They are, however, relatively less sensitive, and quite strikingly so as time goes on, to administered HCG as judged by the response obtained in hypophysectomized-cryptorchid animals.

C. A. Paulsen: We would agree. The limited response to HCG in these patients indicates that Leydig cell function is not normal. It is possible that more LH is required to produce a certain level of plasma testosterone. In some patients normal levels are attained, whereas in others testosterone levels remain low.

K. W. Thompson: With regard to the total LH output in the urine of patients with this condition, only one such has come my way, but we did follow the total urinary LH output for about 15 days. At the same time, we were doing two normal males. The level of LH per day in the Klinefelter's case was the same high level as that group of erratically high women that I mentioned last night. The level was totally irregular, and about 3 or 4 times the level of the normal men who showed continuously regular levels. The Klinefelter's case did not show any change in LH output after a single intramuscular dose of 100 mg of testosterone propionate. Those women with high erratic levels do show a dramatic drop with Orthonovum. In one single day there will be a sharp drop. In one an intramuscular dose of 100 mg of progesterone caused a very sharp rise in LH output. Presently we are starting this Klinefelter's man on testosterone enanthate to see whether or not a longer period of testosterone administration will bring the LH down.

C. A. Paulsen: Even in normal males urinary gonadotropin excretion titers are not suppressed by a single intramuscular injection of 100 mg of testosterone. Therefore, I am not surprised that your patient with Klinefelter's syndrome did not demonstrate a decrease in LH titers.

P. J. Mulrow: This failure of Klinefelter patients to respond to exogenous testosterone with a normal growth of facial hair is not unique for Klinefelter's syndrome. I have been impressed in my limited experience with these patients who had hypogonadism and delayed puberty from pituitary tumors, who, despite large doses of exogenous testosterone, did not develop a normal growth of facial hair. I think that something is happening to the hair follicles.

I would like to ask you a question regarding your clinical description of this syndrome.

3002457

In the literature, a high incidence of mental deficiency has been reported, and I did not hear you mention this in your description.

C. A. Paulsen: We would agree that the problem of refractory facial hair growth is not confined to patients with Klinefelter's syndrome. Our experience is similar to yours in that we have observed this phenomenon in patients with hypogonadotropic eunuchoidism and pituitary tumors. To my knowledge there are no data available which define the basic mechanisms involved. It may be that the patient's age at the start of treatment is just as important as genetic factors in altering the response of the hair follicles to exogenous androgen.

We did not cover the problem of mental deficiency since there was not sufficient objective data in our patients to bear on the issue we were emphasizing, namely the influence of XY/XXY mosaicism. There are, of course, ample data which document a higher incidence of Klinefelter's syndrome in mentally retarded patients as contrasted to the general population.

A. B. Fajer: In relation to the high levels of LH in blood, I would like to refer to another situation in the constant estrous, androgen-sterilized rat. In our Department, Dr. Barraclough has shown that these animals secrete very small amounts of estrogen and have very large amounts of LH in plasma and when these animals are castrated and the estrogen is removed, these levels of LH fall to the values seen in normal, castrated animals. Dr. Barraclough was able to show a separation between the action of very small amounts of estrogen, really minute amounts of estrogen, on the release of estrogens from the pituitary compared to the storage of LH in the pituitary, in opposition to large amounts of estrogen that always blocked the release of LH to the plasma than those required in the castrated animals. I wonder how this can be translated in terms of testosterone and LH. Maybe there is a similar situation, or perhaps there is a little bit of estrogen. I don't know.

C. A. Paulsen: In patients with Klinefelter's syndrome urinary estrogen excretion is variable [C. A. Paulsen, "Estrogen Assays in Clinical Medicine," Univ. of Washington Press, Seattle, Washington, 1965]. Some patients exhibit entirely normal titers while others demonstrate low values. But I am unable to translate the animal data which you referred to into the hormonal pattern found in these patients. At this time all we can say is that the pituitary-gonadal axis in Klinefelter's syndrome may be different. The details await further study.

A. F. Parlow: I showed in my presentation one slide which depicted serum LH and FSH levels in patients with Klinefelter's syndrome. For comparison the data from normal males and surgical castrates were included. LH levels were highest in the surgical castrates but definitely increased in patients with Klinefelter's syndrome. These data may bear on the question of testosterone LH interrelationship.

C. A. Paulsen: Your data demonstrate that there is a difference between LH levels in patients who exhibit some degree of testicular function and those who are completely devoid of androgen secretion. Since we have no data in surgical castrates we were not able to make a direct comparison.

H. H. Swanson: I would like to make a comment similar to that of Dr. Lloyd about the lack of reactivity to testosterone possibly being due to a lack of testosterone during fetal life. Rats and hamsters castrated neonatally do not react to exogenous testosterone normally.

The other comment I would like to make concerns the explanation for the gynecomastia. I think you mentioned that the estrogen level was normal. I wonder whether

3002458

gynecomastia also may be related to the early influence of hormones. Rats castrated neonatally or receiving androgen show development of mammary glands at about 1 week of age which is seen in normal females but not in normal males. The lack of mammary development in males may be a matter of estrogen-sensitive primary tissue not being laid down. Although the patients have normal estrogen secretion in adult life, they may, like neonatally castrated animals, react more like females to the same level of estrogen.

C. A. Paulsen: No one has the answer to the pathogenesis of gynecomastia. In my opinion it has been a mistake to focus almost all attention on estrogen metabolism in such patients. When you consider in what conditions gynecomastia is encountered, it seems to me that the pituitary is involved. For example, gynecomastia is seen in normal puberty and various types of hypogonadism where pituitary gonadotropins are elevated or at least present. It is rare to see gynecomastia in untreated patients with hypogonadotropic eunuchoidism. Perhaps prolactin secretion stimulates the male breast and is primarily responsible for gynecomastia in conditions where estrogen levels are clearly normal.

C. W. Lloyd: Returning to this problem of androgen response, Dr. Mulrow raised a point that made me get to my feet. That is the question of failure of hair growth in certain disease states in which there may be failure of other pituitary target organs and the relationship of response to androgens to steroid or other hormones present. The Japanese, who normally have very little hair, have normal plasma testosterones, but it is interesting that their 17-ketosteroids are considerably below those of Caucasians of the same body mass and the same surface area. We wondered, then, whether there might be something that is a precursor to the 17-ketosteroids that might be related to this change in hair. Dr. E. Klaiber and I have demonstrated that in the castrated, immature, male rat given a standardized minimally effective dose of testosterone, as far as producing seminal vesicle and prostate enlargement if you add cortisol to this you can double the effect. So it looks as though cortisol is augmenting androgen. In treating hirsute women, we find that some who have elevated testosterones and who get worse when you give them a corticosteroid don't have the drop in testosterone that you expect. We think that in these people the corticosteroid may be augmenting the testosterone. Now I don't think this would apply in Klinefelter's syndrome in the sense that you have no evidence of any adrenal defect. But, I think this might be a factor in some other individuals in whom there is pituitary failure and who perhaps have some adrenal aberration. I wonder if, perhaps, in the Klinefelter's we may have two choices, or maybe both apply. One is a lack of fetal imprinting of androgen responsive tissues, and perhaps another one is that the subjects do not have other factors they ought to have to make the testosterone work in the usual way.

L. M. Sherwood: Dr. Swanson has already raised some of the points I was going to mention; namely, the question whether gynecomastia might be related to some pituitary factor or whether it is dependent on the estrogen-androgen balance. To confuse the problem a little further, I have had recent experience with one patient with Klinefelter's syndrome who developed gynecomastia which regressed with oral methyltestosterone therapy and then reappeared with cessation of the therapy. This might suggest that the gynecomastia is due to a relative increase in the estrogen to androgen ratio, rather than some pituitary factor.

C. A. Paulsen: Methyltestosterone which is not converted to estrogenic substances *in vivo* can produce gynecomastia in some hypogonadal patients. Other androgens have this ability also.

A. Segaloff: I wonder whether you have any observation on common baldness in

3002459

treated patients who do not develop facial hair. With reference to the possibly inappropriate LH levels, I wonder, if one were to assume that the LH which is measured by the radioimmunoassay and by most of our biologic assays is part of the FSH molecule and if you were to correct for the LH from the FSH, whether indeed these people would not have normal or even low LH values and this therefore would mean that it is not really an inappropriate level.

C. A. Paulsen: Since we do not have direct comparison between serum FSH and LH, investigation of correction factors between these two gonadotropins cannot be made.

With respect to your question on baldness, we have recently seen two patients with familial baldness who had hypogonadism. One patient was an example of Klinefelter's syndrome (W.H.); the other had hypogonadotropic eunuchoidism (H.G.). In W.H. frontal baldness developed after he was treated with small doses of methyltestosterone. Facial hair growth was not stimulated by this form of therapy. In H.G. baldness developed before androgen therapy. Thus androgens may be permissive with respect to baldness in some patients but not in others.

M. M. Grumbach: I suggest caution in the use of the term "inappropriate LH secretion," especially in the manner in which we have applied it in this discussion. The point Dr. Tait raised is an important one. We do not know the plasma concentration of free (non-protein-bound) testosterone in these patients. It is possible that, when measured, it will be low. Further, impaired Leydig cell function with a limited capacity to secrete testosterone may be an important factor.

C. A. Paulsen: The method for plasma testosterone measures unconjugated testosterone, therefore it is true that the relationship between "bound" and "free" testosterone is not known.

M. M. Grumbach: The values you reported represent testosterone extracted from plasma, but the proportion of protein-bound to free plasma testosterone has not been studied.

J. F. Tait: I am not sure I followed your argument that your data showed that the male adrenal contributed androstenedione. Was that solely based on the androstenedione values not going down in some of these subjects?

C. A. Paulsen: We were referring to studies by other investigators such as yourself. The data on plasma androstenedione were shown to document that patients with Klinefelter's syndrome demonstrated normal levels. Thus the testicular contribution was not increased.

J. F. Tait: There is still a possibility though, isn't there, that the testes in these subjects may switch to make androstenedione, as in the prepubertal male?

C. A. Paulsen: Yes, I suppose so.

I. Rothchild: I think it is rather amusing that in a collection of scientists like us so much of the discussion has revolved around the question of the "inappropriateness" of the LH levels and testosterone levels. These are very sick testes and there may be many things that they are not doing that normal testes would be doing, and these may have a great deal to do with the secretion of LH, FSH, and perhaps other pituitary hormones. We are looking only at the concentration of testosterone in the blood stream, which is probably only one of a dozen parameters of normal testicular function. The much more fascinating question concerns the relationship between the chromosome defect and the abnormal testes, and this is particularly striking in the mosaic Klinefelter's because there's a stem-line of spermatogonia with normal chromosomes and another stem-line with abnormal chromosomes. Now why does the testis *as a whole* behave

3002460

abnormally? You can see spermatogenesis going up to spermatid development, and perhaps in a few individuals up to spermatozoa. But why does it stop there, and why is it so inefficient up to that point? Why don't the XY cells go all the way up the line to normal spermatozoa? I don't know the answer. I am just raising a question. We know from studies of the spermatogenic cycle that each cellular element in the cycle is probably related to the others, and all of these are related in some way to the activity of the Sertoli cells. The really interesting question then is: What is it that's wrong about the XXY stem-line that disturbs what should be a normally functioning XY stem-line of spermatogonia?

C. A. Paulsen: That is the 64 dollar question. How does an overdosage of X-chromosomes produce testicular disease? Another point that requires consideration is the relationship between XY/XXY mosaicism and the uneven pathology encountered in the testes. Is it possible that the two stem-cell lines are distributed so that in areas where spermatogenesis is more normal the XY line predominates? Or are the two stem-cell lines distributed evenly throughout the testis and the variation in pathology related to some other factor? I know of no way to study this problem in a critical fashion.

Originally, we thought it might be possible to predict the degree of damage by knowing the percentage relationship between the XY and XXY stem-cell lines. But as mentioned in the paper, it isn't as simple as that.

C. G. Heller: The question of gynecomastia has been raised several times during the presentation and discussion and I'm happy to see the interest being taken in it. I would like to point out why the Paulsen series has more gynecomastia than any other series. Paulsen answered it only partly when he mentions the complete physical examination. Actually, Paulsen himself is like Avis—'He tries harder.' Indeed in instances when I've concluded that there was no gynecomastia, and Dr. Paulsen has examined the same patient he has said, "Certainly there's gynecomastia—let me show you." And he's been able to demonstrate this and I think it would behoove some of us to be more careful and to try harder too!

One of the coauthors of the classical paper by Klinefelter *et al.* [H. G. Klinefelter, Jr., E. C. Reifenstein, Jr., and F. Albright, *J. Clin. Endocrinol.*, **2**, 615 (1942)], is present—Dr. Reifenstein. There is a mistake in that paper regarding gynecomastia. I don't know whether Dr. Reifenstein is aware of it, but their illustration purporting to be gynecomastia in Klinefelter's syndrome is not that at all. It is of normal male breast tissue. Since little is written about either the histology of normal male breast tissue or of gynecomastia in Klinefelter's syndrome, I call your attention to this oversight.

N. T. Werthessen: I would like to raise the question: Has anyone studied, in this syndrome, the applicability of Lyon's hypothesis of the inactive X chromosome in the normal female? We have here two X's and one Y. Are there any data to indicate that one of these becomes inactive as it does in the normal female? Would failure to do so explain some of the testicular phenomena and the peculiar behavior of various portions of the anatomy?

C. A. Paulsen: The presence of a condensed X-chromosome in these patients is indicative of inactivation. Tritiated thymidine studies have also been compatible with this. I cannot recall any special studies that have been carried out. Perhaps Dr. Grumbach can add to this point.

M. M. Grumbach: There is no evidence that the presence of a Y chromosome affects the inactivation of a second or greater number of X chromosomes. Just as in the normal human female, a large part of one of the two X-chromosomes in the somatic cells of

3002461

XXY individuals is heterochromatinized and gives rise to the Barr body. In some cells it is the matroclinous X and in others the patroclinous X.

N. T. Werthessen: I see. But have you evidence here as in those sex-linked G6PD deficiency states of the erythrocytes that were used to prove the applicability of the Lyon's hypothesis to man?

M. M. Grumbach: The same type of evidence has been obtained in XXY individuals. For example: erythrocyte glucose-6-phosphate dehydrogenase activity in XXY individuals is the same as that in XO and XY individuals and not increased, suggesting suppression of the X-linked gene directing erythrocyte G6PD synthesis on one of the two X-chromosomes.

N. T. Werthessen: If there is suppression of *an* X then the double X could not explain the anatomical aberration and the abnormal testicle.

M. M. Grumbach: The point is that in man, in contrast to the mouse, inactivation of the second X in terms of a dosage effect, is not complete. The fact that XO and XXY human beings are not normal is one among several pieces of evidence indicating the X-inactivation is incomplete in man. Several hypotheses have been advanced to explain the testicular lesion and certain other clinical findings associated with an XXY karyotype, but these are speculative. Nevertheless, it is suspected that these phenomena are related at least in part to a gene dosage effect from the extra sex chromosome.

E. E. Baulieu: Is there any indication that there could be some abnormality of the peripheral metabolism of testosterone in Klinefelter's syndrome, especially the oxidative catabolism? It could have some impact on the plasma testosterone level and on the activity of testosterone in so-called target organs.

C. A. Paulsen: To my knowledge, such studies have not been carried out. There have been a few studies on the metabolism of androgens by male mammary skin. These were normal males as I recall. Work in this area seems to be just beginning.

3002462

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