CHAPTER IV

SECRETION & EXCRETION OF STEROIDS IN KLINEFELTER'S SYNDROME

& HERMAPHRODITISM

Klinefelter's syndrome is associated with hypogonadism and is characterised by seminiferous tubule failure and adenomatous clumping of Leydig cells. In this chapter adrenocortical function of four patients suffering from Klinefelter's syndrome and one case of hermaphroditism has been studied by determining urinary 17-KS, 17-KGS and plasma corticosteroids. Since Klinefelter's syndrome is associated with the presence of 'positive' chromatin pattern, sex chromosomal configuration has been discussed along with hormonal investigation.

RESUME OF LITERATURE

(A) Klinefelter's syndrome

Nelson was the first to indicate that Klinefelter's syndrome may be associated with a female type of sex chromatin in anatomically male subjects. This theory was subsequently confirmed by Plunkett and Barr, Bradbury, Grumbach et al, Riis et al, Jackson et al and Wisochi et al. The patients suffering from this disease have atrophic testes with hyalinization of seminiferous tubules and clumping of Leydig cells. They suffer from azoospermia. Because of gonadal dysgenesis, they hardly secrete testosterone, thus the 'feed back' mechanism is not present and as a result, high urinary concentrations of gonadotrophins are observed.
Grumbach et al. studied the sex chromatin pattern extensively in such patients and observed that in male subjects the presence of female sex chromatin associated with testicular pathology indicated this disorder to be of congenital origin. It was also shown that the disorder occurred in males having XY or XXY chromosomes. The clinical feature of Klinefelter's syndrome, as originally described, was a common manifestation of seminiferous tubules dysgenesis. However, not all patients with seminiferous-tubules dysgenesis have Klinefelter's syndrome. They further explained that the failure of the embryonic gonad to differentiate as an ovary in chromosomal females with seminiferous tubules dysgenesis, could be attributed to a severe corticomedullary imbalance with complete suppression of the cortical component which is the ovarian primordium. As a result, the embryonic gonad is transformed into a testis, capable of bringing about male sex differentiation. Recently, Sohval has reviewed extensively the sex-chromosome configuration in patients suffering from Klinefelter's syndrome to establish the correlation between sex-chromatin patterns, sex-chromosome constitutions and testicular function.

(B) Hermaphroditism

The sex development in placental mammals consists of the differentiation of the gonads, the genital ducts and the external genitalia. Though very little is known about the control of the differentiation, the unidirectional course of future gonadal differentiation is predetermined by the
genetic sex of the zygote, established at the moment of fertilization. Sex determination is a genetic phenomenon and established before sex differentiation by the combination of parental sex chromosome. It is understood that the physiological reactions controlled by the balance of these sex-determining genes, effect the differentiation of the bipotential gonad to either testis or ovary. Early in the procedure of embryogenesis, the embryonic gonad is composed of two distinct unipotential mesodermal primordia, a cortex consisting of the germinal epithelium and a medulla made up of the primary sex cords and the mesonephric or blastemal elements. The sex specificity of the medullary and cortical structures in vertebrates is a well established physiological and anatomical concept. In the human, the different gonads can be identified during the fifth week of embryonic life, though its morphologic constituents are not as sharply delineated as in the lower vertebrates. The germinal elements of the gonad are represented by the primordial germ cell, which originates in the endoderm, and in man these cells migrate to the genital ridge at the time of early gonadal differentiation. The cortex can differentiate only as an ovary and the medulla only as a testis; and during sex differentiation, each of these elements competes for dominance. The dominant element conforms to the genetic sex of the zygote and the recessive element retrogresses. When the gonad begins to develop in a male direction, the cortical component involutes and only limited secondary sex cord formation occurs. However, until the cortical component
completely regresses, the potential for forming ovarian elements persists. If the gonad destined to develop into an ovary, secondary sex cords form the germinal epithelium of the cortex and the medullary component including the primary sex cords recedes. In placental mammals the transition from a sexually different gonad is relatively more rapid than in many lower species, and the recessive component is transient and less well demarcated. The sex differentiation in the gonadal morphosis and also the pathway of future development of secondary sex characters are predetermined at an early embryonic stage. The testes appear distinctly by the seventh week of embryonic life.
MATERIAL

The patients were divided into two groups:

(1) Klinefelter's syndrome
(2) Hermaphroditism

(1) Patients suffering from Klinefelter's syndrome:

A group of four patients, who were apparently males but showed female sex chromatin on microscopic examination of oral smear and testicular biopsy, were investigated for urinary 17-KS and 17-KGS and 17-OHCS in plasma. In three out of four of these cases ACTH infusions were carried out. Patient NW was on testosterone therapy for one year and during this period the excretion of 17-KS was checked from time to time.

(2) Hermaphroditism:

One patient of male pseudohermaphroditism was investigated. This patient (BV) had a penis, one small testis, a well developed uterus and one functioning ovary. The patient was bleeding every 32 days through the urethra. This case was studied over a period of 6 years. This patient had female type sex chromatin. In this patient the urinary 17-KS and 17-KGS, estrogen and pregnanediol and plasma 17-OHCS were determined before and after ACTH infusion.

Patient BV was operated and at his request one ovary and the uterus were removed. Since the patient was
only 14 years old, the testicle was given a chance to develop without treatment. During this period the excretion of 17-KS was observed and also when he was treated with testosterone over a period of two years.

This patient was given HCG, total dosage of 9,000 I.U. intramuscularly and plasma 17-OHCS and the excretion of 17-KS and 17-KGS were studied.
RESULTS

Plasma 17-OHCS, urinary 17-KGS and 17-KS in patients suffering from Klinefelter's syndrome

The values of plasma 17-OHCS in these patients varied from 3.9 to 15.0 mg per 100 ml of plasma. The excretion of 17-KGS and 17-KS per 24 hours ranged from 4.2 to 7.8 and 2.0 to 7.2 mg respectively. (Table XXI). In all these cases the gonadotrophin test was positive at 6.6 and 50 rat units. Similarly, all these four patients showed a female type of sex chromatin.

Plasma and urinary steroids in a case of true hermaphroditism:

This patient had a regular menstrual cycle of 32 days and on the 27th day of the cycle the urinary excretion of 17-KS, estrogens and pregnanediol were 6.3 mg, 211.0 /μg, and 2.1 mg per 24 hours respectively. During a laparotomy in October 1955, ovary was found in the abdomen of the patient which was surgically removed. The urinary estrogen values decreased and the pregnanediol disappeared. There was no change in the excretion of 17-KS following surgery. (Table XXII).

In November 1958, a standard test dose of ACTH was infused to this patient. The patient's plasma 17-OHCS values increased from 13.5 to 45.5 /μg per 100 ml and the excretion of 17-KGS increased from 3.0 to 8.3 mg per 24 hours. The 17-KS
Table XXI

Plasma 17-OHCS, Urinary 17-KGS and 17-KS in patients suffering from Klinefelter's syndrome

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Plasma 17-OHCS µg/100 ml.</th>
<th>17-KGS mg/24 hours</th>
<th>17-KS mg/24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>25</td>
<td>5.2</td>
<td>2.6, 2.0, 3.8</td>
<td>7.4, 7.4, 7.0</td>
</tr>
<tr>
<td>AN</td>
<td>20</td>
<td>5.4, 3.9</td>
<td>4.2, 5.9</td>
<td>4.2, 4.5</td>
</tr>
<tr>
<td>NZ</td>
<td>36</td>
<td>15.0</td>
<td>7.0</td>
<td>5.2</td>
</tr>
<tr>
<td>LK</td>
<td>22</td>
<td>-</td>
<td>7.2, 6.7</td>
<td>7.8, 6.5</td>
</tr>
</tbody>
</table>

Range ...
3.9 - 15.0
4.2 - 7.8
2.0 - 7.2

Mean ...
7.4
5.0
6.3

Gonadotropin test was negative at 6.6 and 50 rat units
A female type sex chromatin was present in all cases.
**Table XXII**

Concentration of urinary and plasma steroids in one case of true hermaphroditism before and after surgical removal of abdominal ovary in August 1955

<table>
<thead>
<tr>
<th></th>
<th>Urinary 17-KS mg/24 hrs.</th>
<th>Urinary Estrogens μg/24 hrs.</th>
<th>Urinary Pregnanediol mg/24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before operation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 1955</td>
<td>6.3</td>
<td>211.0</td>
<td>2.1 27/32*</td>
</tr>
<tr>
<td><strong>After operation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>October 1955</td>
<td>4.6</td>
<td>Not done</td>
<td>0.0</td>
</tr>
<tr>
<td>December 1955</td>
<td>6.0</td>
<td>57.0</td>
<td>0.0</td>
</tr>
<tr>
<td>March 1956</td>
<td>5.1</td>
<td>35.7</td>
<td>0.0</td>
</tr>
<tr>
<td>December 1957</td>
<td>4.6</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Urinary 17-KS mg/24 hrs.</th>
<th>Urinary 17-KGS mg/24 hrs.</th>
<th>Plasma 17-OHCS μg/100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 1958</td>
<td>6.7</td>
<td>3.0</td>
<td>12.5 Control levels</td>
</tr>
<tr>
<td>Nov. 1958</td>
<td>7.6</td>
<td>8.3</td>
<td>42.5 After 25 IU ACTH</td>
</tr>
<tr>
<td>Nov. 1958</td>
<td>5.8</td>
<td>2.4</td>
<td>12.8 Control levels</td>
</tr>
<tr>
<td>Nov. 1958</td>
<td>6.4</td>
<td>4.5</td>
<td>Not done 1 day post 3000 IU HCG I.M.</td>
</tr>
<tr>
<td>Nov. 1958</td>
<td>5.8</td>
<td>2.5</td>
<td>Not done 1 day post 6000 IU HCG I.M.</td>
</tr>
<tr>
<td>Nov. 1958</td>
<td>8.0</td>
<td>2.0</td>
<td>Not done 1 day post 9000 IU HCG I.M.</td>
</tr>
<tr>
<td>Nov. 1958</td>
<td>5.2</td>
<td>4.5</td>
<td>Not done 2 day post 9000 IU HCG I.M.</td>
</tr>
</tbody>
</table>

* On 27th day of 32 days menstrual cycle.
increased slightly. In the same month 9,000 I.U. of HCG were injected intramuscularly and the excretion of 17-KS and 17-KGS were measured at three stages during HCG administration. The results are given in Table XXII.
A Klinefelter's syndrome:

The patients investigated in the present work were phenotypically males. All these patients noticed sexual abnormalities at puberty. They were fairly nourished but their musculature was poor. Patient NW and AN had gynaecomastia with a good amount of glandular tissue. All of these patients had accumulation of feminine type of fat and none of them had beard or hair on the chest, and only scanty axillary and pubic hairs were present. The size of penis and testicular mass was small in all the cases.

The microscopic observation on their buccal mucosa suggested the presence of sex chromatin mass ordinarily present in females i.e. a positive sex chromatin. On examination, the testicular tissue showed hyalinisation of seminiferous tubules with clumping of Leydig cells. In patient ND the Leydig cells were infrequently seen.\(^\text{15}\)

Repeated assay of urinary gonadotrophins indicated high secretion of these hormones in all cases. The plasma content of 17-OHCS, the urinary 17-KGS and 17-KS excretion were within the lower limits established for normal man. Patient NR excreted a consistently low concentration of
17-KGS as shown in Table XXII. The present investigation has added to our understanding of Klinefelter's syndrome that such patients have normal adrenals which will respond to ACTH in a normal way. It is therefore, highly unlikely that the adrenal cortex in Klinefelter's patient is responsible for the development or the maintenance of this syndrome.

B Hermaphroditism:

The patient (BV) was 14 years old, apparently a male with one testicle but showed in addition, breast development, high pitch voice and accumulation of gynaecoid fat. He complained of regular menstrual bleeding through a penile urethra. The X-ray examination revealed the female type of pelvic girdle. The buccal mucosa and the skin biopsy contained 'female' sex chromatin. The urinary values of 17-KS, estrogens and pregnanediol were similar to those found in normal woman of active reproductive age.

The patient experienced occasional erection of penis and the ejaculation fluid did not contain sperms. These clinical and biochemical data suggested the presence of an ovary or ovaries. On laparotomy, a well developed uterus with fallopian tube and one ovary was found. The vagina was opening into penile urethra. As the patient was brought up as a male and psychologically felt like a male, the female gonad and uterus was surgically removed. Following surgery, the menstrual bleeding and the excretion of pregnanediol disappeared.
Also the urinary estrogen excretion was reduced to the normal level exhibited by normal men. No change in the excretion of 17-KS was, however, observed.

The histological studies of the surgically removed ovary in this patient indicated the presence of corpus luteum and all characteristics of a normal functioning ovary. The testis was small in volume and histological examination on testicular biopsy revealed no germinal cells and undeveloped seminiferous tubules. As the patient was only 14 years old and the source of estrogens was removed, the testicle was allowed to develop without therapy. Three years after the surgical removal of the ovary, the 17-KS excretion was still unchanged. The 17-KGS excretion was lower than normal and the concentration of plasma 17-OHCS was normal. After ACTH administration, the plasma 17-OHCS and the excretion of 17-KS and 17-KGS were increased indicating the presence of normal adrenals. 9,000 I.U. of HCG was injected intramuscularly over a period of 8 days and no increase of excretion of 17-KS or 17-KGS was observed. This indicated that the testicular tissue was not stimulated by HCG. Finally, this patient was given testosterone propionate 100 I.U. every 15 days. After this treatment he grew facial hair and his voice became coarse.

The etiology of this disorder lies in the sex differentiation in the intra uterine life. The fetus might
have developed from a zygote composed of XX chromosomes, which means that the genital sex of the fetus was female. At the time of sex differentiation the cortex of one of the primordial bodies developed into an ovary as controlled by the genital sex while in the other primordial body, the medulla might have developed instead of regressing, giving rise to testis and the child was born with one descended testis and one abdominal ovary. The history of the pregnancy of the mother of patient does not indicate any hormone therapy during pregnancy. The reason for the anomaly in this patient is still unknown.
SUMMARY

1. The patients suffering from Klinefelter's syndrome had female type of sex chromatin and they secreted high amounts of gonadotrophin.

2. The adrenocortical function in these patients was normal.

3. a) A case of hermaphroditism was genetically and anatomically a female with a penis and a testis. The excretory levels of various steroids were similar to those of a normal woman. The patient had normal adrenal function.

   b) After surgical removal of the ovary, the testicular tissue did not develop and did not respond to exogenous HCG.

   c) The etiology of this disorder has been discussed.
REFERENCES


