the first day of treatment; (2) the effect of the treatments on the size of the spleen during the first three years; (3) the haemoglobin concentration during the first two years; and (4) the total leucocyte and platelet counts after the first course of treatment.

Three years after the entry of the last patient into the trial 30 of the 48 patients in the busulphan group (62.5%) and 18 of the 54 patients in the radiotherapy group (33.3%) were alive.

The median survival of the busulphan group was 170 weeks and of the radiotherapy group 120 weeks after the start of treatment; 20% of the busulphan group survived 263 weeks and 20% of the radiotherapy group 182 weeks.

In the radiotherapy group the median survival was superior in males, the values for the busulphan (26 male patients) and radiotherapy (26 male patients) groups being 170 and 127 weeks respectively, compared with 170 and 114 weeks respectively for the females in the busulphan (22 patients) and radiotherapy (28 patients) groups.

Busulphan and radiotherapy were equally effective in reducing the size of the spleen in the initial course of therapy, and there was little difference in their efficacy in controlling the tendency of the spleen to enlarge during the first three years. The spleen was impalpable for varying periods during each of the first three years in 50% of the patients in each group (30 busulphan, 18 radiotherapy) who survived three years.

Busulphan was superior in causing the haemoglobin concentration to rise to arbitrarily chosen "normal" levels. Only one of 48 patients failed to reach the lower range of normal levels, compared with seven of 54 patients in the radiotherapy group.

Busulphan was significantly better than radiotherapy at maintaining the haemoglobin concentration within an

arbitrarily chosen normal range, the probability of the difference being due to chance being less than 0.00003 in the case of males and 0.02 in the case of females.

Busulphan was also more effective than radiotherapy in sustaining normal haemoglobin levels during the second year, the probability of the difference being due to chance being less than 0.01.

The leucocyte and platelet counts at the end of the first course of treatment continued to fall in a similar proportion of patients in both groups, but the fall continued, on average, three weeks longer in the busulphan group.

When the trial was ended 90 patients had died. Death resulted from blast-cell transformation in 70% of the patients. The incidence was the same in both treatment groups, but the transformation arose earlier in the radiotherapy group.

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Secretion of Androgens and Oestrogens in Testicular Feminization: Studies in Vivo and in Vitro in Two Cases*

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Patients with so-called testicular feminization have a female body habitus with apparently well-developed breasts, a lack of body hair being a marked feature. There is no uterus, and the gonads are testes situated within either the abdomen or the inguinal canal. They present superficially as females with primary amenorrhoea. These observations led to the term "testicular feminization" being adopted for these patients (Morris, 1953). The sex chromosome pattern is a normal male one, 46 XY (Jones, 1965); the condition is familial, being inherited, probably as a sex-linked recessive.

Despite the early supposition that the abnormality in testicular feminization lay in the testis, which was producing oestrogen rather than androgen, it has been known for some years that urinary 17-oxosteroid and oestrogen excretions are in the ranges for normal men (Morris, 1953). Griffiths *et al.* (1963) reported the first in-vitro study on a testis which showed the ability to form androstenedione and testosterone, but not

oestrogen. In the same year Morris and Mahesh (1963) reported the isolation of testosterone from testicular venous blood in the condition.

In the present study androgen and oestrogen secretion in two patients with testicular feminization has been assessed by the measurement of production rates in vivo by isotope dilution, by synthesis in vitro from ¹⁴C-progesterone, and in one case by the concentration of steroids in testicular vein plasma and of free testosterone in peripheral plasma.

Case Histories

Case 1.—This patient was aged 17. A "sister," two years older, also had testicular feminization. At operation one gonad was found in the abdomen, the other in the inguinal canal; both were removed. Buccal squames were chromatin-negative.

Case 2.—This patient was aged 16. A sister, one year younger, was normal. At the age of 6 the patient had had a herniorrhaphy, and an "ovary" had been found in the inguinal canal. Ten years later both gonads were within the abdomen, and were removed.

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Buccal squames were chromatin-negative and the chromosome count of the white cells showed a single population, 46 XY.

Histology of Cases.—The testes from both patients showed hyperplasia of the interstitial cells—marked and diffuse in Case 1 but rather patchy in Case 2. The tubules were mostly uncanalized and lined by Sertoli cells. Very occasional spermatogonia were also seen in the testes in Case 1.

Methods

Total C_{19} -steroid, testosterone, and oestrogen production rates were measured by isotope dilution in urine after the injection of ^{14}C -testosterone and ^{14}C -oestrone (Case 1) or 3H -oestradiol (Case 2). The methods used for purifying the radioactive metabolites have been described elsewhere (Jeffcoate *et al.*, 1968b). Production rates in Case 1 were measured under basal conditions. In Case 2 production rates and peripheral plasma testosterone concentration were measured while the adrenal cortex was suppressed with dexamethasone. In addition, observations were made during gonadal stimulation with: (1) Pergonal in a daily dosage containing 225 i.u. of follicle-stimulating hormone (F.S.H.) and 225 i.u. of luteinizing hormone (L.H.) (it should be noted that in spite of the unitage the biological activity of this preparation with respect to L.H. is very low); (2) a combination of Pergonal with human chorionic gonadotrophin (H.C.G.) (5,000 i.u. daily). Plasma testosterone was also measured after gonadectomy during dexamethasone suppression plus H.C.G. stimulation. The detailed schedule is given in the Diagram.

Schedule for production rate determination in Case 2 before and after gonadotrophin stimulation. PR=Production rate. Op=Operation (gonadectomy)

Venous blood was collected from the testicular vein at laparotomy in Case 2 and the plasma separated by centrifugation. The latter was then analysed as described for ovarian vein plasma (Jeffcoate *et al.*, 1968a).

Slices of tissue (0.5 mm. thick) from the testes of both patients were incubated in vitro with ^{14}C -progesterone (5 μCi) as substrate. Incubations were carried out at 37° C. in Krebs-Ringer bicarbonate buffer under an atmosphere of O_2/CO_2 (95/5) for two hours. Cofactors were not added to the medium, as this might have distorted the synthetic activity of the glands. After addition of cold carriers (200- $\mu g.$ amounts) of androstenedione, testosterone, oestrone, and oestradiol, the fractions were purified and specific activities measured by the methods described elsewhere (Jeffcoate *et al.*, 1968a). Peripheral plasma testosterone was measured by the method of Lim and Brooks (1965).

Results

The results of C_{19} -steroid, testosterone, and oestrogen production rates in these two cases are shown in Table I; the normal ranges for these rates in both men and women are given in Table II.

In Case 1 the C_{19} -steroid and testosterone production rates were both in the male range. The oestrogen production rate

was at the lower end of the normal female range and within that for normal men.

In Case 2 also the testosterone production rate was in the male range; there appeared to be a small increase with Pergonal, but a much bigger one after H.C.G. was also given. The oestrogen production rate was low and not increased by Pergonal, but it was greatly increased by the addition of H.C.G. The plasma testosterone was above the normal female range but rather lower than was expected on the production

TABLE I.— C_{19} -steroid, Testosterone, and Oestrogen Production Rates (mg./day) and Plasma Testosterone ($\mu g./100$ ml.) in Two Patients With Testicular Feminization

Case No.	Treatment	Production Rates (mg./day)					Plasma Testosterone (mμg./100 ml.)
		C ₁₉ -steroid	Testosterone	Oestrogen from Specific Activity of			
				Oestrone	Oestradiol	Oestriol	
I	Control	30.5	12.1	0.06	0.06	0.09	—
2	I Dex.	7.2	6.3	0.03	0.02	0.02	126
	II Dex. + Pergonal	9.8	7.6	0.03	0.02	0.02	104
	III Dex. + Pergonal + H.C.G.	10.3	10.8	0.36	0.15	0.45	235
	PO Dex. + H.C.G.	—	—	—	—	—	16

For treatment schedule in case 2, see Fig. 1 and text. Dex. = Dexamethasone.

PO = Postoperative (gonadectomy).

TABLE II.—Normal Ranges for C_{19} -steroid, Testosterone, and Oestrogen Production Rates (mg./day) and Plasma Testosterone ($\mu g./100$ ml.) in Men and Women

		Production Rates (mg./day)			Plasma Testosterone ($\mu g./100$ ml.)
		C_{19} -steroid	Testosterone	Oestrogen	
Men	{ US S	15-40* <10*	4-12†	0.03-0.1	670-1096**
Women	{ US S	8-25* <6*	0.8-2.9‡ 0.4-2.0§	0.04-0.3¶	24-90**

US = Unsuppressed. S = During adrenal suppression.
* Calculated from 17-oxosteroid excretion in normal subjects assuming that 40% of secreted C_{19} -steroid excreted as 17-oxosteroids (Baulieu and Mauvais-Jarvis, 1964; McSwiney *et al.*, 1964).

† Korenman *et al.* (1963).

‡ Hudson *et al.* (1965); Korenman *et al.* (1965).

§ Korenman *et al.* (1965).

|| Morse *et al.* (1963); Lipsett *et al.* (1966).

¶ Goering *et al.* (1965).

** Lim and Brooks (1965).

rate results. There was no increase with Pergonal, but again a definite increase with additional H.C.G. After gonadectomy the plasma testosterone was very low even during H.C.G. administration.

The results of the in-vitro incubation of testicular slices with ^{14}C -progesterone are shown in Table III. Testosterone was the major product found. Synthesis of oestrogen was low and not even definitely established.

TABLE III.—Percentage Conversion in vitro of ^{14}C -progesterone into Androstenedione, Testosterone, and Oestrogens by Testicular Tissue in Two Patients with Testicular Feminization

Case No.	Androstenedione	Testosterone	Oestrone	Oestradiol
1	0.36	0.98	(0.0005)	(0.001)
2	(0.07)	0.21	(0.0005)	(0.0008)

Figures in parentheses indicate that constancy of specific activity was not established.

A sample of testicular vein blood (3.4 ml.) was obtained from Case 2 and the concentration of steroids ($\mu g./100$ ml.) found was: testosterone, 9.0; androstenedione, 3.1; dehydroepiandrosterone, <0.5; oestrone, <1.2; oestradiol, <1.2. For comparison the concentration of testosterone in normal male testicular plasma is 12-119 $\mu g./100$ ml. (Dupré *et al.*, 1964) and of androstenedione 0.8-7.7 $\mu g./100$ ml. (Jeffcoate *et al.*, 1967). In normal ovarian vein plasma the levels found in one study (Horton *et al.*, 1966) were: 0.05-0.10 $\mu g./100$ ml. for testosterone and 0.45-1.20 $\mu g./100$ ml. for androstenedione.

Levels of oestradiol in ovarian vein plasma were between 1.1 and 2.8 in three normal women (Mahesh, 1965).

Discussion

These results confirm the findings of others that both *in vivo* and *in vitro* the testes in testicular feminization are synthesizing steroids in a relatively normal fashion with male levels of both androgen and oestrogen secretion. The levels of testosterone glucuronide in urine have been found to be those of normal men (Rosner *et al.*, 1965). The present study confirms that so also are the testosterone concentrations in testicular vein plasma (Morris and Mahesh, 1963; French *et al.*, 1966). The male levels of testosterone found in peripheral plasma by French *et al.* (1965, 1966) were not found in one patient in this study, being unexpectedly low considering that the testosterone production rate was over 6 mg. daily. The testosterone production rate has been measured by only one other group (Southren *et al.*, 1965b), and found, as here, to be in the male range. The production rate of 12.1 mg./day observed in Case 1 is high even for a man, and it is of interest that the testes in this case showed marked and diffuse hyperplasia of the interstitial cells. The oestrogen production rates, not previously measured in this condition, were also consistent with male levels. Some of the limitations of the measurement of production rates by isotope dilution have been discussed elsewhere (Jeffcoate *et al.*, 1968b).

The findings *in vitro* of a major conversion of progesterone to testosterone, and, to a less extent, to androstenedione, with very low oestrogen formation are in accordance with the observations of Griffiths *et al.* (1963), Morris and Mahesh (1963), David *et al.* (1965), Kase and Morris (1965), and Southren *et al.* (1965a).

All these studies strengthen the view that the testes in testicular feminization are functioning normally so far as steroid secretion is concerned. This is further supported by the observations on the effects of stimulation of the testes with gonadotrophins. It is doubtful if F.S.H. alone stimulated steroid production, but the addition of H.C.G. greatly increased the secretion of testosterone and of oestrogens, owing to its L.H. activity. The increase in oestrogen secretion was similar to that reported in normal males (Lipsett *et al.*, 1966). If the Pergonal alone had any effect at all it could have been due to the small proportion of L.H. present. It is clear that H.C.G. was, as expected, without effect after gonadectomy, since the peripheral plasma of testosterone was virtually zero when the blank value of the method is taken into account (Lim and Brooks, 1965).

Summary

The gonadal formation of androgen and oestrogen in two patients with testicular feminization has been investigated *in vivo* and *in vitro*.

Male levels of testosterone secretion were suggested by the urinary production rates of testosterone, the testosterone levels in peripheral and testicular venous plasma, and the synthesis of testosterone *in vitro*.

Secretion of oestrogen was low, as judged by the low oestrogen production rates, and the failure to detect oestrogen in testicular vein plasma or to establish a definite synthesis *in vitro*.

H.C.G. greatly increased the secretion of testosterone and of oestrogens in one patient. The results strengthen the view that the defect in the disease lies outside the testis, which is secreting steroids in a relatively normal male fashion.

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