THE STIMULATION AND PROLONGED MAINTENANCE OF SPERMATOGENESIS BY HUMAN PITUITARY GONADOTROPHINS IN A PATIENT WITH HYPOGONADOTROPHIC HYPOGONADISM

F. I. R. MARTIN
Endocrine Clinic, Royal Melbourne Hospital, Melbourne, Australia

(Received 30 December 1966)

SUMMARY
The clinical course of a man with an apparently isolated deficiency of pituitary gonadotrophin is described. Despite interstitial cell stimulation, prolonged therapy with human chorionic gonadotrophin did not produce spermatogenesis. In contrast, 60 days after treatment with human pituitary gonadotrophin had started, viable spermatozoa were seen and spermatogenesis was subsequently maintained for 16 months.

The sequence of changes observed supports the concept that the action of follicle-stimulating hormone in the testicular tubule is at a late stage of spermatozoa formation.

INTRODUCTION
In recent years there have been several reports of the use of human gonadotrophin to stimulate spermatogenesis in the treatment of complete gonadotrophin deficiency following total hypophysectomy (Gemzell & Kjessler, 1964; Macleod, Pazianos & Ray, 1964; Macleod, Pazianos & Ray, 1966) or craniopharyngioma (Lytton & Kase, 1966). In addition Lunenfeld, Mor, Mani & Zinberg (1965) have described the successful treatment of azoospermia by human gonadotrophins in men with 'nil or absent gonadotrophins', and Heller (1965) produced spermatozoa in a man with hypogonadotrophic hypogonadism by treatment with pituitary gonadotrophin and human chorionic gonadotrophin.

This report describes the clinical course of a man with hypogonadism due to an isolated deficiency of pituitary gonadotrophin production who, at the age of thirty-one, was treated with human chorionic gonadotrophin (HCG) for 12 months and then with both human pituitary gonadotrophin (HPG) and HCG for 22 months.

CASE REPORT
The patient first came to the Endocrine Clinic of The Royal Melbourne Hospital in 1956 at the age of 24 because of 'failure of normal male development'. He was of northern Italian origin and on examination appeared typically hypogonadal with a
high pitched voice, small testes and penis and very little body hair. Libido was absent and he did not shave. Although his height was 64 in. (163 cm.) he was of eunuchoid proportions with a span of 69½ in. and a lower segment measurement of 34 in. No other abnormalities were found; at this time bone age was estimated by X-ray to be 17-18 yr. and the pituitary fossa appeared normal. Testicular biopsy was refused and treatment was started with testosterone propionate i.m., 50 mg./week. This resulted in normal penile development, the appearance of body hair but very little facial hair, and growth to a final height of 64½ in. He was maintained on testosterone, 30 mg./day sublingually for 7 yr. During this time he married and worked as a labourer. Libido and potency were normal and satisfactory except when he discontinued testosterone for a 2-months trial. Because of concern with fertility several seminal analyses were performed at other laboratories during this period and all were said to show azoospermia. In 1963 at the age of 31 he was fully investigated because he was dissatisfied with his infertility, his youthful appearance and lack of facial hair. Examination showed a stocky, well built man with very little facial hair but axillary and pubic hair of female distribution. The penis was of normal adult size, the scrotum rugose, both testes were small and soft, measuring approximately 1-5×2 cm. He complained of long standing indigestion and an X-ray examination revealed a chronic duodenal ulcer. After withdrawal of androgen therapy for 4 weeks investigations showed: Total urinary gonadotrophins less than 4-0 mouse units (m.u.)/day on two occasions, weak positive, 2-5 m.u./day, on one occasion. The value in this laboratory for normal men is 5-45 m.u./day (Martin, 1964), and one unit by the mouse uterus assay is equivalent to 25-40 µg. second IRP. Basal urinary 17-oxosteroids were 6-7 and 10-0 mg./day respectively. After metyrapone, 500 mg. 6 hourly for 48 hr., 17-hydroxysteroid excretion was 15-5 mg./day. Thyroid function and insulin tolerance were normal. Chromosome analysis of peripheral leucocytes showed a normal male complement of 46 chromosomes. Testicular biopsy was performed under general anaesthesia. The histological section (Plate, fig. 1) showed well-formed tubules lined by two or three layers of cells. Mitoses were present but scarce, and it was considered that the cells represented primary and secondary spermatogonia with some spermatocytes. There was an almost complete absence of interstitial cells and no evidence of fibrosis.

Total urinary oestrogens were 3-0 and 3-1 µg./day on two occasions and the plasma testosterone level was 0-05 µg./100 ml. Both values are well below the normal male range for the methods used which were those of Brown, Bulbrook & Greenwood (1957) for oestrogens (normal range 7-9-19-6 µg./day) and Hudson, Coghlan, Dulanis, Wintour & Ekkel (1963) for testosterone (normal male range 0-4-0-95 µg./100 ml.). A diagnosis of isolated hypogonadotrophic hypogonadism was made and it was decided to begin treatment with HCG (Pregnyl, Organon), 5000 i.u. twice a week. After 1 month libido and potency were much increased and the plasma testosterone had risen to 0-33 µg./100 ml. Total urinary oestrogen excretion was 13-8 µg./day (Table 1). After a further 4 weeks during which 2500 i.u. HCG was administered twice a week, libido and potency, plasma testosterone (0-62 µg./100 ml.) and urinary oestrogens (7-5 µg./day) were all normal. However, there was no significant change in the excretion of urinary 17-oxosteroids and 17-hydroxysteroids and azoospermia persisted with an ejaculate of 0-5-1-0 ml. After 3 months HCG was discontinued to
Spermatogenesis with HPG in hypogonadism

see whether spontaneous puberty had been induced. There was a rapid decline in libido and potency and after 4 weeks the total urinary oestrogens and plasma testosterone had fallen to 3-9 µg./day and 0-35 µg./100 ml.; urinary gonadotrophins were assayed as less than 4-0 m.u./day.

Treatment with 2500 i.u. HCG once a week was recommenced in September 1963 and continued for 9 months until June 1964. Seminal analyses performed monthly showed azoospermia in volumes of 0-5 to 1-5 ml., except on two occasions after treatment for 5 and 7 months respectively, when two or three abnormal, non-motile sperm were seen in the ejaculate. Clinically, the testes had not significantly altered after this period of treatment and in June 1964 a second testicular biopsy was performed. The histological appearance (Plate, fig. 2) of the tubules was very similar to the previous biopsy. There were still only two or three layers of cells within the tubules, although mitoses were more frequent and more obvious. No spermatids or spermatozoa were seen despite careful examination of a number of sections by different observers. In contrast, interstitial cells were much more numerous and obvious and there was a slight increase in the fibrous elements of the interstitial tissue.

After 4 weeks without HCG the patient had again lost libido; plasma testosterone concentration was 0-28 µg./100 ml. and urinary oestrogen excretion 3-2 µg./day, urinary gonadotrophins were just detectable at 3-0 m.u./day, and seminal analysis still showed azoospermia. Treatment with HPG was then started. The preparation used initially was obtained by the method of Steelman, Segaloff & Mays (1958) from acetone-dried pituitary powder. Subsequently HPG was prepared by Dr J. B. Brown by the method of Brown, Catt & Martin (1967). The initial dose used was approximately equivalent to 500 i.u. of FSH (as assayed against the second IRP by the mouse
ovarian augmentation assay) per week given as two injections of equal dose. In addition, to ensure adequate androgenic stimulation, 1,000 i.u. HCG was given once per week. The subsequent course during the next 22 months is shown in Text-fig. 1.

Seminal analyses were performed every 2 weeks and after 61 days of treatment motile, morphologically normal spermatozoa were seen for the first time at a concentration of approximately 140,000/ml. Sixty-eight days after the commencement of therapy the dose of HPG was increased to 750 i.u./week as a thrice weekly injection. This regime was maintained for a further 9 months. The effects on spermatogenesis and hormone production are shown in Text-fig. 1 and Table 1.

Table 1. Oestrogen excretion and plasma testosterone concentrations in a patient with hypogonadotrophic hypogonadism during treatment with human chorionic gonadotrophin (HCG) and human pituitary gonadotrophin (HPG)

<table>
<thead>
<tr>
<th>Treatment, dosage/week (i.u.)</th>
<th>Date</th>
<th>Daily oestrogen excretion (µg.)</th>
<th>Plasma testosterone concentrations (µg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>7. ii. 63</td>
<td>2.4 0.6 0.3 3.0</td>
<td>-</td>
</tr>
<tr>
<td>HCG, 10,000</td>
<td>9. ii. 63</td>
<td>1.9 0.9 0.4 3.1</td>
<td>0.05</td>
</tr>
<tr>
<td>5. v. 63</td>
<td>16. iv. 63</td>
<td>6.5 0.8 1.5 13.8</td>
<td>0.33</td>
</tr>
<tr>
<td>Nil (4 weeks)</td>
<td>4. iv. 63</td>
<td>2.9 1.0 0.2 3.9</td>
<td>0.35</td>
</tr>
<tr>
<td>HCG, 2000</td>
<td>15. vii. 63</td>
<td>11.0 6.1 1.8 18.9</td>
<td>-</td>
</tr>
<tr>
<td>HCG, 2500</td>
<td>7. vi. 64</td>
<td>11.5 4.1 0.0 15.6</td>
<td>-</td>
</tr>
<tr>
<td>Nil (3 weeks)</td>
<td>7. vi. 64</td>
<td>2.1 1.0 0.0 3.2</td>
<td>0.28</td>
</tr>
<tr>
<td>HCG, 1000</td>
<td>18. vii. 64</td>
<td>5.8 3.2 2.7 11.7</td>
<td>-</td>
</tr>
<tr>
<td>HPG, 500</td>
<td>14. ix. 64</td>
<td>3.8 1.9 1.9 7.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Ditto for 2 months</td>
<td>17. i. 65</td>
<td>1.7 3.6 1.8 7.1</td>
<td>0.46</td>
</tr>
<tr>
<td>HCG, 1000</td>
<td>15. 5. 65</td>
<td>2.9 1.6 1.0 5.5</td>
<td>0.74</td>
</tr>
<tr>
<td>HPG, 750</td>
<td>12. i. 66</td>
<td>- - - -</td>
<td>0.79</td>
</tr>
</tbody>
</table>

The sperm count rose steadily to a level of 25,000,000 8 months after treatment had started. The volume of ejaculate varied from 1.5 to 2.5 ml., the motility from 30 to 70 % and the percentage of abnormal forms from 10 to 50 % on different occasions.

In July 1965, 12 months after commencing treatment it was decided to double the dose of HPG to approximately 1500 i.u. FSH/week. This was done because there seemed to be a slight decline in the seminal count at this time, and because the patient’s wife had not become pregnant although her menses were regular and ovulatory by temperature chart. After 1 month on this increased dose, HCG was withdrawn because of concern that peritubular fibrosis might have been induced and treatment with HPG was continued alone at a dose of 1500 units/week for a further 2 months. The sperm count rose to the previous peak level and was maintained with an ejaculate of 2.5–3.0 ml., motility 30–50 % and abnormal forms 10–20 %, but while off HCG libido decreased and treatment with 500 i.u. HCG/week was re-started which provided adequate stimulation. At the same time, since no apparent effect on spermatogenesis had been observed with the increased dose, HPG was reduced to 500 i.u./
Spermatogenesis with HPG in hypogonadism

week. This regime was continued for a further 10 weeks with maintenance of spermatogenesis at much the same level as before but a decrease in ejaculate to 1.5–2.0 ml. During the whole of this 15-month period of treatment the patient and his wife had failed to achieve pregnancy despite careful advice and satisfactory intercourse, and both began to show features of anxiety which included exacerbation of the patient’s epigastric pain and an irregular anovulatory menstrual cycle in his wife.

Since the continuation of treatment was placing a severe strain on the available supplies of HPG, the possibility that maintenance could be achieved by one injection per week instead of two or three was considered. The total urinary gonadotrophin excretion for the day following injection of both 250 units HPG and 500 units HCG was 30 m.u. a day by the mouse uterus assay. The results of an assay repeated 5 days after injection was not significantly different with a level of 25 m.u./day suggesting that an adequate level of gonadotrophin had been maintained during this period.

After discussion with the patient, who wished to continue treatment, it was explained to him that it was intended to reduce the dose of HPG to the minimum required for maintenance. Treatment with 250 units HPG and 500 units HCG once per week was then begun and appeared to maintain spermatogenesis, as a seminal count of 18,000,000/ml. was obtained 4 weeks later. Treatment was then altered to 250 units HPG without HCG and the excretion of total gonadotrophin in the urine was measured on the day after injection and 6 days later. The gonadotrophin levels found were 48 m.u. and 32 m.u./day respectively, again suggesting continued gonadotrophin activity during this period. For this reason this dosage was continued, but after 6 weeks the patient complained that his libido had decreased, and he was unable to provide a seminal sample for examination. Methyltestosterone, 30 mg./day sublingually, was then substituted for HCG and treatment with HPG continued at 250 i.u. FSH/week. Six weeks later the patient reported improved potency but seminal analysis of an ejaculate of 9.0 ml. revealed almost complete azoosperma with only approximately 250,000 non-motile spermatozoa/ml. Treatment with HCG (1000 i.u./week) and HPG (250 i.u. twice weekly) was restarted and the administration of testosterone discontinued. The result of a seminal analysis 1 week later was unchanged, but when repeated after 6 weeks 1.5 million spermatozoa/ml. were seen in a volume of 1.5 ml. with 60% motility and 20% abnormal forms. The patient continued on this regime but did not report back for further examination and has subsequently decided to discontinue treatment although still requesting androgen replacement. Throughout the whole period of treatment clinical examination did not show any significant alteration in testicular size, although beard growth appeared to have gradually increased over 3 years.

DISCUSSION

The diagnosis of isolated pituitary gonadotrophin deficiency as the cause of hypogonadism appears valid, since the patient was of normal height with no evidence of adrenal or thyroid deficiency, and total urinary gonadotrophins without treatment were either absent or at very low levels on several occasions. Despite the fact that detectable, but minimal gonadotrophic activity was found in the urine on two occasions both before and after HCG therapy, libido and potency were only main-
tained by either exogenous androgens or injections of HCG. In addition, both the plasma testosterone and urinary oestrogen levels were very low without treatment so that the functional significance of the very low gonadotrophin excretion is doubtful. The sequence of events observed indicates that HCG stimulates Leydig cells to produce both testosterone and oestrogens (predominantly oestradiol) but does not stimulate spermatogenesis significantly beyond the stage of spermatocyte formation. The appearance of a few non-motile spermatozoa in the semen during HCG stimulation is unexplained, but could be related to the presence of very low levels of endogenous pituitary gonadotrophin. Although histological proof was not obtained, the appearance of motile sperm in the semen approximately 60 days after starting treatment with an 'FSH rich' extract of human pituitary glands agrees with the observations of Heller & Clermont (1964) and Macleod et al. (1966) who suggest that in man FSH stimulates the later stages of spermatozoa formation and possibly meiosis and spermatid production. It seems that stimulation was maximal with a dose of 750 units HPG/week since doubling this dose for 3 months did not produce an increase in spermatogenesis. Maintenance of spermatogenesis by less than 500 i.u. HPG/week may have been possible, since the excretion of total urinary gonadotrophins was maintained for 6 days at apparently normal male levels after one injection of 250 i.u. HPG, but, after 10 weeks on this dose, during which time methyltestosterone was required to maintain androgenic function, almost complete azoospermia had occurred. The relationship between the changes in treatment and the failure of spermatogenesis at this stage was unfortunately not completely documented. However, the observations suggest that in the treatment of hypogonadism caused by failure of pituitary gonadotrophin production it is necessary to give both chorionic gonadotrophin at doses of 500–1000 i.u./week and pituitary gonadotrophin twice weekly to maintain spermatogenesis. In a recent description of a similar case, Johnsen (1966) also concluded that both HCG and human menopausal gonadotrophin were necessary for spermatogenesis.

This report illustrates that it is possible to stimulate spermatogenesis with pituitary gonadotrophins to a level of potential fertility in a man with hypogonadotrophic hypogonadism of long duration. Despite the continued stimulation for almost 3 years which seemed to achieve maximal sperm production there was no significant increase in testicular size. The treatment was stopped partly because the supply of HPG available was limited, but also because the prolonged therapy caused considerable anxiety in both the patient and his wife. It is important that the stress effects that such a regime may produce be continually kept in mind.

The author is grateful to Professor Bryan Hudson, Department of Medicine, Monash University, Prince Henry's Hospital Melbourne and Dr J. B. Brown, Department of Obstetrics, University of Melbourne Royal Women's Hospital Melbourne, for the performance of the plasma testosterone and urinary oestrogen assays, respectively. Mr G. Senator supplied the photomicrographs.

Human pituitary gonadotrophin and human chorionic gonadotrophin (Pregnyl, Organon) were the gifts of the Victorian Pituitary Committee and British Drug Houses of Australia Ltd., respectively.
REFERENCES


DESCRIPTION OF PLATE

Staining: haematoxylin and eosin; magnification: × 240.

Fig. 1. Testis (first biopsy), February 1963, before treatment.

Fig. 2. Testis (second biopsy), June 1964, after therapy with human chorionic gonadotrophin for 12 months.