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SUPPRESSION OF HUMAN SPERMATOGENESIS BY DEPOT ANDROGEN: POTENTIAL FOR MALE CONTRACEPTION

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SUMMARY

The gonadotropic hormones, LH and FSH, are required for normal sperm production. Inhibition of gonadotropin secretion by exogenously administered sex steroids represents one possible approach for male contraception. In the present study testosterone enanthate (TE) was administered IM to 39 normal adult men (age 21–39) to assess its efficacy as a suppressor of spermatogenesis and to determine possible adverse effects. Seventeen subjects (Group A) received TE (200 mg/wk) for 16–20 weeks. 16/17 lowered sperm counts to less than 5 million/cc; 11/17 to less than 300,000; and 10/17 became azoospermic. Group B received TE (200 mg/2 wk). In 10/22, sperm counts were less than 5 million/cc at 16 weeks; 9/22 were less than 300,000; and 5/22 were azoospermic. When those with sperm counts greater than 5 million/cc were switched at 16 weeks to weekly treatment (additional 3–16 weeks), 9/12 lowered sperm counts to less than 5 million/cc. Overall, 19/22 of Group B attained this level. Serum LH and FSH were decreased on both regimens. These effects were dose-related. Mean serum testosterone was elevated above baseline (+64%) at 1 week after injection (Group A), but remained at basal levels 2 weeks after TE injection in Group B. Serum oestradiol levels parallel those of serum testosterone. Decreasing the frequency of TE (3 or 4 weeks) resulted in a rebound of FSH and LH above baseline and increased sperm counts. After discontinuing treatment, sperm counts and hormonal measurements returned to normal in all subjects. Modest increase in body weight, red cell mass, oiliness of skin and mild acne were seen in some subjects. Liver function tests, glucose tolerance, blood lipids and renal function were unchanged. Based on these data, testosterone enanthate demonstrates significant suppression of spermatogenesis. Further refinement of dose and/or delivery system, as well as investigation of combination hormonal regimens show promise for the development of a safe, effective male contraceptive agent.

INTRODUCTION

Population expansion is an issue of great concern throughout much of the world. Natural resources and available foodstuffs have lagged behind the increasing numbers of people, particularly in the economically emerging and underdeveloped countries. Progress produced by the development of oral contraceptives for women during the 1950's and 60's has proven to be insufficient for all needs. Inflation and changing lifestyles for women have resulted in increasing numbers of families with two wage earners and have raised our consciousness of the need for new and better approaches to family planning. Recent concern over side effects from oestrogen-progestogen containing oral agents taken by women and a growing desire of many men to share the responsibilities of family planning have increased interest in the development of effective and safe male contraceptive agents. Such agents could substitute for the irreversible vasectomy and the unesthetic or inadequately effective use of condoms or coitus interruptus.

The hormonal control of spermatogenesis is a complex and incompletely understood process. Both LH and FSH are required for initiation of maturational events; and high intratesticular testosterone levels are required for maintenance of germinal maturation [1]. Interference with the process of sperm maturation is one potential mechanism for controlling fertility in the male. Since androgens, oestrogens and progestins will suppress gonadotropin secretion and spermatogenesis in both animals and humans [2–10], some investigators have considered the use of testosterone either alone or in combination with progestogens or oestrogens as a male contraceptive agent [11–21].

Preliminary studies by a number of investigators [22–29] have suggested that depot forms of testosterone could be administered in dose regimens that reversibly suppress both gonadotropins and spermatogenesis. This study will evaluate the efficacy, mechanism of action and adverse reactions of several dose regimens of testosterone enanthate given for the purpose of inhibiting spermatogenesis in the male. The effects of the dose regimen on semen, reproductive hormones, secondary sexual organs and selected specific laboratory and clinical parameters have been evaluated.

MATERIALS AND METHODS

Volunteer subjects

Fifty-three volunteer male subjects, 21 to 39 years of age, participated in the study. Each was informed

of the nature of the study; all were believed free of major systemic or psychiatric disease and had normal physical examinations; none were on medications known to influence reproductive function. Before entry each was screened for abnormalities of reproductive hormones (LH, FSH, testosterone and estradiol), semen analysis, liver function (LDH, SGOT, alkaline phosphatase, bilirubin, prothrombin time, albumin and globulin), blood count (CBC), glucose (fasting blood sugar), lipids (triglycerides and cholesterol), electrolytes (Na, K, Cl, HCO_3, Ca, and P). Of the 53 original subjects, 14 did not complete either the control phase or the initial treatment phase. None of the 14 excluded subjects were eliminated because of adverse effects during the study. Only the 39 subjects completing at least the initial phase of treatment were included in the analysis.

Protocol

The study was divided into four study periods: (1) a control phase lasting 8 weeks; (2) treatment phase 1, lasting 16 or more weeks; (3) treatment phase 2, lasting 24 weeks; and (4) a recovery period that lasted until all laboratory tests returned to normal.

During the control phase, baseline hormonal, toxicological, and semen measurements were obtained at two week intervals for two months. Upon entry into the first treatment phase, the subjects were divided into two groups (A and B). Group A (17 subjects) received testosterone enanthate (200 mg intramuscularly) every week, while group B (22 subjects) received the same dose every 2 weeks. Hormonal measurements and semen analyses were obtained every 2 weeks just before the next testosterone injection. All subjects who had suppressed their sperm counts to below 5 million/cc on two consecutive occasions by 16 weeks of treatment phase 1 entered into phase 2 of treatment. Those subjects in Group B who had sperm counts greater than 5 million/cc at 16 weeks were placed on weekly treatment for an additional 16-week period. Subjects were entered into phase 2 as soon as they met the above criteria for sperm counts. Some of the subjects in Group A who did not meet the criteria for entry into phase 2 were continued on weekly treatment for an additional 1–4 weeks. All subjects failing to suppress on the above regimens were placed in the recovery phase.

Upon entry into treatment phase 2, the subjects were randomized into treatment groups with the same dose of testosterone enanthate (200 mg) every 3 or 4 weeks. Blood samples were obtained both every 2 weeks and just prior to the next testosterone injection. This report presents only preliminary data on the subjects in phase 2 and recovery phase of the study.

Semen Analyses

Seminal fluid samples were obtained every 2 weeks by masturbation following at least 2 days of abstinence. Semen volume was recorded; sperm counts, motility and morphology were determined using standard techniques.

Hormone measurements

Serum LH, FSH, testosterone, and oestradiol were measured by radioimmunoassay [30–34] on samples obtained by combining equal volumes of three separate serum specimens obtained 20 min apart. Cross reaction of testosterone enanthate in the testosterone assay was less than 0.5%. This finding is similar to that of Caminos-Torres *et al.*[35]. Inter and within assay variations in our laboratory have been characterized: LH ($\pm$10.7% and $\pm$6.6%); FSH ($\pm$12.1% and $\pm$3.3%); testosterone ($\pm$12.4% and $\pm$6.7%); and oestradiol ($\pm$12.4% and $\pm$5.7%). Data has been corrected for between assay variations using computer analysis of multiple pooled control samples.

CBC and toxicologic screening

These were performed once monthly using automated Coulter Counter and M-300 procedures. Glucose tolerance testing was performed once in each experimental period.

Physical examinations

A complete physical examination was performed once monthly. Body weights and vital signs were carefully recorded. Testes size were quantified using a Prader orchidometer; each testis was measured and the mean size of the two calculated.

Data analysis

All the data from each of the control subjects were entered into a computer file and stored on magnetic tape using IBM/360 APL language. The different treatment groups were handled separately. Means, standard errors of the mean, percent change from control, statistical testing and correlation analysis were computed. A separate set of plotting routines were used to generate the graphs.

RESULTS

(1) *First phase of treatment*

Mean serum testosterone, oestradiol, LH and FSH for the subjects receiving weekly and bimonthly treatment are shown in Figs 1 and 2. The mean $\pm$ SEM are plotted for the 8 week control period and the 16 week treatment period (phase 1); N = 17 weekly and 22 bimonthly subjects. Serum testosterone was elevated during weekly testosterone administration compared to control (662 $\pm$ 32 vs 427 $\pm$ 25 ng/dl) (Fig. 1). In contrast, bimonthly treatment (Fig. 2) produced no changes in serum testosterone when measured 2 weeks after the last injection. Changes in serum oestradiol concentrations paralleled those of testosterone. Weekly treatment resulted in a mean increase of serum oestradiol from 27.2 $\pm$ 1.5 to 42.5 $\pm$ 3.6 pg/ml at 14–16 weeks (Fig. 1). The mean

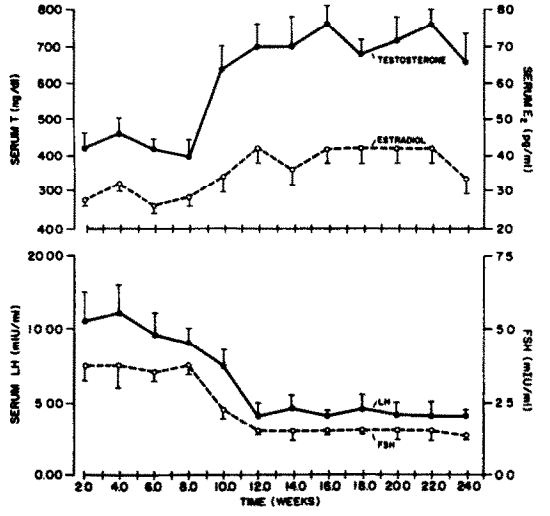

Fig. 1. Mean serum LH, FSH, T and E_2 in 17 subjects on weekly treatment with 200 mg of testosterone enanthate. Control values are seen on the left and the first 16 weeks of treatment on the right.

individual increment was 58.9 ± 14.4%. With bimonthly treatment (Fig. 2) mean serum oestradiol concentrations (2 weeks after the last injection) were lower than baseline at 14–16 weeks (20.7 ± 1.8 vs 27.3 ± 2.2 pg/ml; $P < 0.05$). The mean individual decrement of E_2 at the end of phase 1 was 19.6 ± 6.5%.

Serum LH concentrations decreased after 4 weeks of weekly treatment and stayed at this decreased level throughout the 16 weeks of treatment (Fig. 1). The mean control LH was 10.1 ± 1.3 mIU/ml and the mean LH during treatment was 4.1 ± 0.9 mIU/ml. The mean individual percentage decrease was 57 ± 6%. Following bimonthly treatment, serum LH concentrations decreased at 4 weeks and remained suppressed throughout the treatment (Fig. 2). Mean

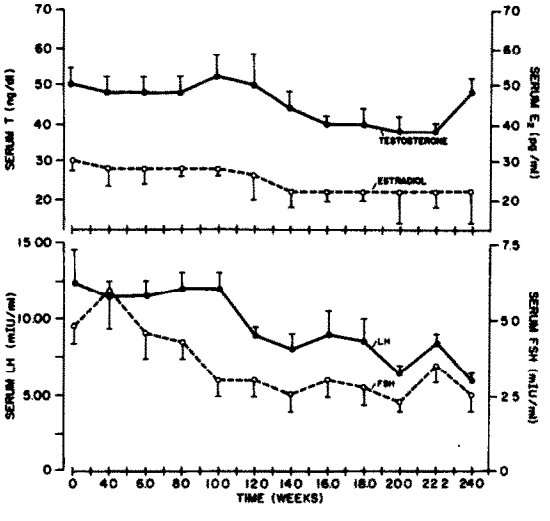

Fig. 2. Mean serum LH, FSH, T and E_2 in 22 subjects on bimonthly TE treatment. Serum samples were obtained just prior to the next injection.

control and treatment phase LH levels were 11.5 ± 0.8 and 7.5 ± 0.9 mIU/ml respectively. The mean individual percentage decrease was 28 ± 12%.

Serum FSH concentration decreased within 2 weeks of initiating weekly treatment and remained suppressed throughout the 16 weeks of phase 1 (Fig. 1). The mean control FSH concentration was 3.69 ± 0.46 and the mean treatment FSH was 1.31 ± 0.13 mIU/ml. The mean individual percentage decrease was 58 ± 2%. In the bimonthly treatment, serum FSH concentrations decreased at 2 weeks and remained suppressed throughout the first treatment phase (Fig. 2). The mean control FSH decreased from 4.7 ± 0.6 to 2.9 ± 0.3 mIU/ml during weeks 14 and 16 of treatment. The mean individual percentage decrease was 14.9 ± 17%. (Several subjects had low control values which contributed to the large variability.)

Mean sperm counts for all subjects receiving weekly and bimonthly treatment are shown in Figs 3 and 4.

Four weeks after beginning weekly testosterone enanthate administration sperm counts decreased below control levels (Fig. 3). By the end of the 16 weeks of treatment, 12 of 17 subjects had sperm counts below 5 million/cc, 11 of 17 had sperm counts below 300,000/cc and 10 of 17 were azoospermic on two consecutive occasions (Table 1). The mean sperm count decreased from a control value of 78 ± 8 × 10^6/cc to 5 ± 2 × 10^6/cc during weeks 14 and 16. The mean individual percent decrease was 94 ± 2%. In five subjects whose sperm counts did not suppress below 5 million/cc at 16 weeks, 1–4 weeks of further weekly treatment resulted in sperm counts below this level in four of the five. One subject did not suppress to below 5 million/cc and was placed in the recovery phase.

Mean sperm count of the 22 subjects receiving bimonthly treatment are shown in Fig. 4. Although the sperm count decreased in all subjects (mean individual percent decrease 73 ± 6%), only 10 of 22 had

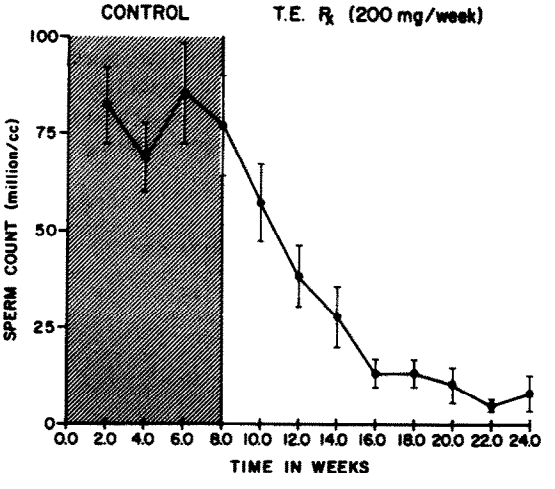

Fig. 3. Mean sperm count in 17 subjects on weekly treatment. (From Swerdloff *et al.* 1977, with permission).

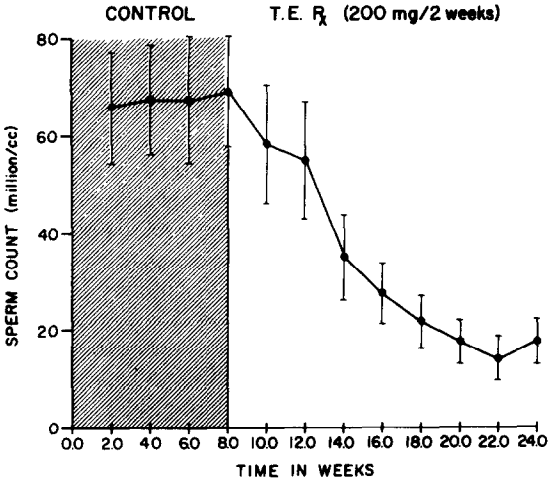

Fig. 4. Mean sperm count in 22 subjects on bimonthly treatment. (From Swerdloff *et al.* 1977, with permission).

sperm counts below 5 million/cc on two consecutive occasions by week 16 (Table 1). Nine of the 22 lowered sperm counts to less than 300,000/cc and only five of 22 were azoospermic. The 12 subjects who did not suppress their sperm count below 5 million/cc after 15 weeks of treatment were placed on weekly treatment and 9 of 12 sperm counts fell below 5 million/cc after an additional 3–16 weeks. Three of these subjects became azoospermic.

(2) *Second phase of treatment and recovery*

Only preliminary data is available on these phases of the study. The experience of nine subjects who were placed on 3 weekly treatment and nine subjects who were placed on 4 weekly treatment following suppression of their sperm count to less than 5 million/cc on two consecutive occasions are presented below.

Serum testosterone, sperm counts, serum LH and FSH concentrations in those subjects receiving testosterone enanthate every 4 weeks are shown in Fig. 5. The data are similar for the 3 week regimen (not shown). The values for phase 1 were obtained after 14–16 weeks of treatment with either weekly or bimonthly testosterone enanthate injections. Data from Groups A and B (phase 1) are combined in this figure; serum hormone measurements during phase 2 of treatment represent the mean of blood samples obtained 3 weeks after injection in the 3 weekly group (not shown) and 2 and 4 weeks after the last injection in the 4 weekly treatment group. Time in weeks of phase 2 refers to the number of weeks after the last injection during phase 1.

In Fig. 5, the value for serum T for phase 1 is not elevated above control. This is the result of combining data from subjects who were receiving either weekly TE (elevated serum T) or bimonthly TE (slightly below baseline). During the second phase of treatment (when the frequency of testosterone enanthate administration was decreased to either 3 or 4 weeks), mean serum testosterone levels remained at or close to pretreatment levels.

Sperm counts were markedly suppressed during the first phase of treatment, began to increase after 10–12 weeks of 3 or 4 weekly treatment, and reached their highest level after 15–21 weeks. Mean sperm counts were at control levels during most of the recovery phase; all subjects attained sperm counts on two consecutive specimens at or above pretreatment levels.

Mean serum LH concentration increased from the suppressed level (end of the first treatment phase) to approximately pretreatment concentrations beginning as early as 4 weeks after the last injection in phase 1 and remained at this level 4 weeks after subsequent testosterone enanthate injections through the 17th week of phase 2. Mean serum LH concentrations measured 2 weeks after each injection during the first 18 weeks of phase 2 (6, 10, 14, 18 weeks on the figure) were slightly lower than those obtained just prior to the next testosterone enanthate injection, giving a cyclic appearance to the LH data. LH levels increased further between weeks 20 and 24 of phase 2 and remained approx. 3 times pretreatment levels. These elevated levels were maintained during the first 6–8 weeks of the recovery period, decreasing to control levels during the last 10 weeks of the study.

On the 4 week treatment regimen, serum FSH concentrations increased from the suppressed values during the first 12 weeks of less frequent therapy, reaching pretreatment levels at approx. 24 weeks of phase 2. As for serum LH, serum FSH values demonstrated a cyclic pattern with lower concentrations seen 2 weeks as compared to 4 weeks after an injection. This response is consistent with partial inhibition of gona-

Table 1. Sperm count in treatment phase 1

Rx	Weeks Rx	< 5 million/ml	< 300,000/ml	Azoospermic
200 mg TE/week	16	12/17	11/17	10/17
	17–20	16/17	11/17	10/17
20 mg* TE/2 weeks	16	10/22	9/22	5/22
200 mg TE/week	17–32	19/22	10/22	8/22

* This group consisted of the 10 subjects who suppressed their sperm count to less than 5 million per ml at 16 weeks and the 12 subjects who failed to suppress their sperm count on the bi-monthly regimen and were switched to weekly treatment.

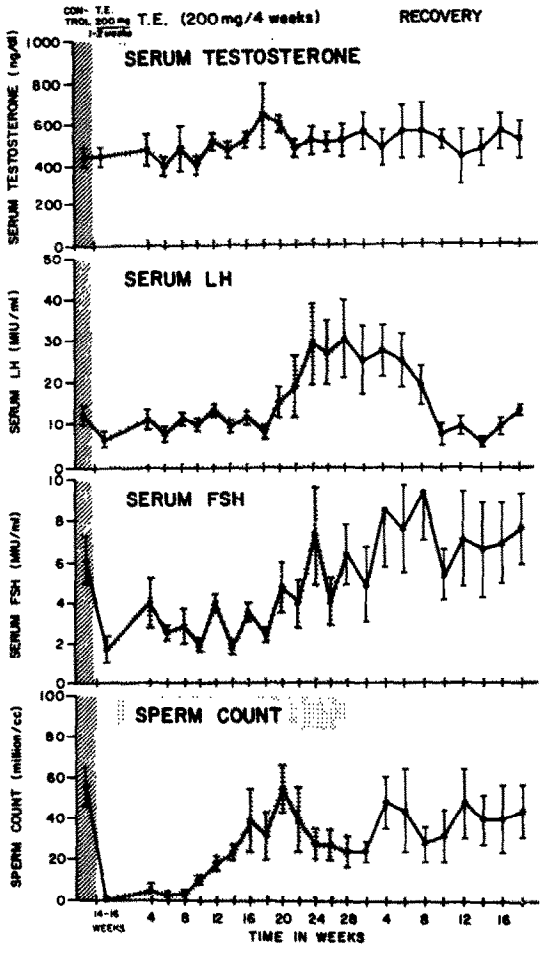

Fig. 5. Mean sperm count, serum testosterone, LH and FSH concentrations in 9 subjects who were placed on 4 weekly treatment following suppression of their sperm count to <5 million/cc during first phase of treatment. The hatched area on the left of the figure represents mean basal levels ± SEM. The clear area labelled week 14–16 represents the mean levels ± SEM during the latter part of phase 1. The shaded area (middle panel) depicts the mean levels ± SEM obtained in phase 2. The clear area to the right of the figure represents the mean levels ± SEM in recovery. The timing of phase 2 refers to the number of weeks since the last injection in phase 1; weeks of recovery represent time since the last injection of phase 2.

dotropins during the integrated periods of either 3 weekly or 4 weekly treatment. Baseline concentrations were maintained throughout most of the recovery period.

Because of the small number of phase 2 subjects involved in this preliminary report, a detailed assessment of the timing of statistically significant rises in serum LH and FSH and their relationship to increases in sperm count must await the inclusion of data from more subjects in the study. Future inclusion of data during phase 2 and recovery from the entire study group will allow separate analysis of the effects of 3 and 4 weekly treatment on subjects who had been treated with either weekly or bimonthly injections during phase 1 of the study.

Toxicology and side effects

Toxicologic data will be presented in detail elsewhere. No significant abnormalities in liver function tests, serum lipid or glucose metabolism were found.

Hemoglobin and hematocrit values increased in most subjects during testosterone administration. This upward trend appears to be dose-related. Despite the statistical increase in hemoglobin and hematocrit values, all subjects except one remained below 18 gm/dl and 52% respectively. Once these individuals started into the second phase of treatment, hemoglobin values gradually returned to control values.

Twenty-five of 39 (64%) subjects reported either increased oiliness of skin or a mild degree of acne. Five of 39 subjects (12.8%) reported a slight increase of hair either on the chest, back or lower abdomen. Twelve of 39 (30%) subjects noted a gain of approx. 3–8% of their body weight.

None of the subjects complained of persistent changes in sexual function. Four of 39 (10%) subjects reported transient breast tenderness and one subject had a mild degree of unilateral breast swelling during bimonthly testosterone enanthate administration that disappeared within 6 weeks of continued treatment.

Thirty-four of 39 subjects (87%) had a decrease in testicular size. The mean decrease in the weekly group (first phase) was 18.3% (Δ of 4.0 ± 0.8 cc). Subjects in the bimonthly group (first phase) had a decrease of 15.3% (Δ of 3.0 ± 0.5 cc). These changes were reversible as evidenced by testicular size returning to control measurements during the recovery period. No alteration was found in prostatic size and consistency.

DISCUSSION

These preliminary data on 39 normal volunteers support the concept that testosterone in doses that produce only modest elevations in serum testosterone levels will suppress gonadotropins and markedly decrease sperm counts to oligospermic or azoospermic levels.

Following weekly treatment with 200 mg of testosterone enanthate, mean serum testosterone was increased less than 2-fold (when measured 1 week after injection) with the majority of values remaining within the usual normal population range. On this treatment regimen, serum LH and FSH decreased rapidly and remained suppressed throughout this phase of treatment. Sperm counts fell as early as 4 weeks and continued to fall throughout the high dose treatment period. The early fall in sperm count suggests that testosterone therapy results in interference either with a late stage of spermatogenesis or with sperm transport. Testes biopsies were not obtained in this study, but the observed decrease in testes size in our subjects and biopsy data from animal [36] and human studies [6] indicate that chronic testosterone treatment does inhibit spermatogenesis. The specific mechanism by which testosterone therapy decreases

sperm counts is of critical importance in development of androgen-containing male contraceptives. It is not known whether inhibition of LH, FSH or both is required for induction of azoospermia.

Correlation analysis yielded a significant relationship between the percent increase in serum testosterone concentration and the percentage decrease in sperm count ($r = 0.56$, $P < 0.05$). When measured 1–2 weeks after testosterone enanthate injection, the correlation of percentage change in LH and FSH concentrations (either alone or in combination) with percent changes in sperm count or testosterone were not significant. Although bimonthly testosterone treatment produced a rapid and sustained fall in both serum LH and FSH, serum testosterone was unchanged. This paradox is due to the fact that serum testosterone was determined 2 weeks after the last injection. Additional data from our laboratories indicate that serum testosterone levels peak as early as 6 h after testosterone enanthate injection and fall rapidly during the first week of treatment reaching baseline levels at approximately the ninth day after injection. Serum testosterone levels are below baseline in many subjects from days 11–14 after injection and may remain lower than control for up to 3 weeks. Preliminary analysis of integrated concentrations of testosterone and oestradiol in the blood of subjects treated either with weekly or bimonthly injections of testosterone enanthate indicate that both regimens result in net pharmacologic levels of blood androgens and oestrogens. More detailed pharmacokinetic studies of serum LH, FSH, and testosterone concentrations following testosterone enanthate injections are required before an understanding of steroid and gonadotropin interactions will be possible. It should be emphasized that total serum testosterone and not free testosterone (unbound) was measured in our study. Since both testosterone and oestradiol influence sex hormone binding globulin levels (in opposite directions), it is difficult to predict whether changes in the biologically free testosterone concentrations would parallel the total testosterone measurements.

Sperm counts decreased slowly during bimonthly treatment until a plateau was reached between 16 and 22 weeks. Correlation analysis revealed no significant correlations among percentage changes in LH, FSH and sperm count. Despite the lack of correlation of percentage decrease of LH and FSH with sperm count, both parameters fell in each subject. Based on the present data we cannot determine if a decrement in LH, FSH, or both is essential for inhibition of spermatogenesis. More detailed analyses of changes in individual subjects are in process. Studies of other investigators demonstrate that this effect of testosterone is reversible with HCG treatment, suggesting that inhibition of LH may play an important role [6].

Our data clearly indicate that the effects of testosterone on spermatogenesis are dose and time related. Weekly treatment was more effective than bimonthly in lowering sperm counts to severely oligospermic levels. Those that did not suppress to less than 5 million/cc at 16 weeks of either weekly or bimonthly treatment had their sperm counts lowered further by either continued treatment or by increasing the frequency of treatment (bimonthly to weekly).

Decreasing the frequency of testosterone administration (200 mg) to every 3 or 4 weeks (phase 2) resulted in failure to maintain the desired sperm count level. On either a 3 or 4 weekly testosterone regimen, sperm counts began to rise after 12 weeks of less frequent treatment. This dose-related effect of testosterone on sperm count has also been reported by others at a recent NIH symposium [27, 29, 37].

The increases in sperm counts seen in treatment phase 2 seemed to follow increases in serum LH and FSH. The elevated levels of serum LH seen in the later part of phase 2 and in the first half of the recovery period were unanticipated and were not correlated with obvious changes in serum testosterone at the times measured. This "overshoot" of serum LH was not associated with further changes in sperm count.

Our data indicate that suppression of spermatogenesis in normal men by testosterone is entirely reversible. Data from others support this concept [27, 29, 37, 38].

Adverse reactions to the administered testosterone were minimal. There were no complaints of impaired sexual function. Increased oiliness of the skin and mild acne were rarely complained of and only detected by careful questioning. Weight gain was observed in 30% of the subjects, but it is not known whether this is due to increased muscle mass, adipose tissue, or secondary to water retention. Hematocrit and hemoglobin levels increased in most subjects, but the increases were modest and were not associated with clinical manifestations.

In conclusion, this report indicates that testosterone enanthate can be given in doses that will suppress sperm counts to below 5 million/cc in most subjects (35 of 39 overall and 16 of 17 with weekly treatment) and produces azoospermia in 36–58% of the subjects. This was accomplished with minimal adverse effects and was entirely reversible. Cunningham *et al.*[37], using a similar 200 mg/wk dose of testosterone enanthate reported preliminary results similar to ours. Steinberger[29] using a somewhat different treatment regimen of testosterone enanthate has reported suppression of sperm count to less than 100,000/cc in 100% of a small series of subjects. While the reasonable goal for a male contraceptive agent would be universal production of reversible azoospermia, it is not known to what degree our subjects would be fertile. It is conceivable that the marked reduction in sperm count seen in all subjects is associated with metabolic alterations in the remaining sperm that prevent fertilization. A model to test this important question is needed. The frequency and mode of administration of testosterone required in this study for optimum suppression of sperm is not ideal for broad

clinical application. Longer acting agents, oral agents, and combining the proper dose of testosterone with other gonadotropin suppressing agents await further evaluation.

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