

EFFECT OF CYPROTERONE ACETATE ON SPERM CONCENTRATION, SEMINAL FLUID VOLUME, TESTICULAR CYTOLOGY AND LEVELS OF PLASMA AND URINARY ICSH, FSH AND TESTOSTERONE IN NORMAL MEN

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(Received 31st December 1971)

Summary. Cyproterone acetate (CA) was administered in a dose of 200 mg/day to six normal men for a period of 16 to 20 weeks. A striking reduction in sperm count, libido and potency was observed, while moderate decreases were detected in testicular germ cell numbers, and testosterone levels in both plasma and urine. No significant change was seen in levels of plasma or urinary ICSH, FSH or in Leydig cell morphology or number. It is concluded that CA is acting directly or indirectly upon the seminiferous tubules to produce a decrease in spermatogenesis which, at least partially, explains the dramatic drop in ejaculate sperm concentration. The observed drop in plasma and urinary testosterone levels was not accompanied by a concomitant drop in plasma or urinary ICSH or Leydig cell number. This could result from an inhibition in testosterone secretion by the action of CA.

INTRODUCTION

Cyproterone acetate (CA) is capable of inhibiting the action of androgen directly and locally at target organs in laboratory animals (Bridge & Scott, 1964; Neumann & von Berswordt-Wallrabe, 1966; Woolman & Hamilton, 1968). In animals, this action can result in feminization of male fetuses (Hamada, Neumann & Junkmann, 1963; Neumann & Elger, 1966a; Neumann, Elger & Kramer, 1966), reduction in size and function of accessory sexual glands (Neumann & von Berswordt-Wallrabe, 1966; Scott & Schirmer, 1966; Neri, Monahan, Meyer, Afonso & Tabachnick, 1967; Beach & Westbrook, 1968; Whalen & Edwards, 1969; Edwards, 1970), a decrease in sebaceous gland volume (Neumann & Elger, 1966b), an inhibition of spermatogenesis (Junkmann & Neumann, 1964; Neumann, Richter & Guenzel, 1965; Neumann & von Berswordt-Wallrabe, 1966; Neumann, von Berswordt-Wallrabe, Elger, Steinbeck, Hahn & Kramer, 1970) and abolition of fertility (Steinbeck, Mehring & Neumann, 1971). No significant change has been detected in

mating behaviour of sexually experienced males (Zucker, 1966; Beach & Westbrook, 1968; Whalen & Edwards, 1969; Whalen & Luttage, 1969), running activity (Stern & Murphy, 1971) or aggression (Edwards, 1970). Testicular interstitial cells of the rat and rabbit have been reported to remain unchanged (Junkmann & Neumann, 1964; Neumann *et al.*, 1965; Neumann, Elger, von Berswordt-Wallrabe & Kramer, 1966; Neumann, Elger, von Berswordt-Wallrabe, 1967; Beach & Westbrook, 1968; Neumann, Elger, Steinbeck & von Berswordt-Wallrabe, 1968; Neumann *et al.*, 1970), or to increase in diameter in the rat (Goslar, Mehring & Neumann, 1970; Heinert & Taubert, 1971).

In man, CA is reported to reduce libido and potency in higher doses (Laschet, Laschet, Fetzner, Glaesel, Mall & Naab, 1967; Ott & Hoffet, 1968), inhibit spermatogenesis (Ott & Hoffet, 1968; Markewitz, Veenema, Fingerhut, Nehme-Haily & Sommers, 1969; Petry, Mauss, Senge & Rausch-Stroomann, 1970), and alleviate the symptoms of prostatic carcinoma without the disadvantages of castration or the occurrence of gynaecomastia (Scott & Schirmer, 1966; Geller, Vazakas, Fruchtman, Newman, Nakao & Loh, 1968; Scott & Wade, 1969).

Because it is hazardous to extrapolate to man the large body of data evolved from animal experiments, our objective was to study the mechanism of action of CA upon the reproductive system of normal men. The usual dose of CA is about 200 mg/day (to treat benign or malignant prostatic hypertrophy). It was decided to use the same dose with normal men to obtain an overall view of its effects on seminal fluid volume, sperm concentration, Leydig-cell and germ-cell numbers, and reproductive hormone concentrations.

If it is true in man, as it apparently is with animals, that CA can reduce fertility without reducing libido or potency (Whalen & Luttage, 1969), then a suitable dose might be found which would serve as a male contraceptive.

MATERIALS AND METHODS

The six subjects in this study were healthy, normal male volunteers between the ages of 23 and 41 years. Judging from testicular biopsy morphology, sperm count, sperm morphology and assay of ICSH, FSH and testosterone, all subjects were reproductively normal.

Routine clinical tests were evaluated before and during CA administration, including blood cytology, urinalysis, plasma calcium, inorganic phosphate, glucose, blood urea nitrogen, uric acid, cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, lactic dehydrogenase and serum glutamic oxaloacetic transaminase.

Cyproterone acetate (6-chloro-17-hydroxy-1 α -2 α -methylenepregna-4,6-diene-3,20-dione acetate; SH 714) was administered orally in a dose of 100 mg twice daily (200 mg/day). The drug was administered continuously for 16 to 20 weeks. Masturbated semen specimens were collected weekly except when unavailable due to emission in the previous 72 hr or lack of potency. Specimens were examined for sperm concentration, motility and volume. Sperm concentration was determined using a haemocytometer and samples were randomly

re-examined for accuracy of counts. Sperm morphology was evaluated on 'blinded' specimens by Dr John MacLeod of Cornell University Medical College according to a method previously described (MacLeod, 1962).

Testicular biopsies were taken 1 to 11 months before CA administration (control), after 3½ to 4½ months of CA administration (administration), and in three subjects, 6½ or 10 months after discontinuation of the drug (recovery). These tissues were surgically removed, fixed in Cleland's fixative and stained with iron haematoxylin and eosin using the method of Rowley & Heller (1966). Germinal cells were quantified from masked slides using the method of Rowley & Heller (1971). In this method, cells were identified according to the criteria of Clermont (1963) and Heller & Clermont (1964). Only those cells cut approximately through the centre of the nucleus were counted so that no correction factor was needed to compensate for cell size. All germinal and Sertoli cells meeting these requirements were counted in thirty cross or longitudinally sectioned tubules. The relative number of each type of germinal cell was expressed as the ratio:

$$\frac{\text{No. of germ cells}}{\text{No. of Sertoli cells}}$$

and is referred to as the 'Sertoli cell ratio'. A germ cell was regarded as abnormal if it appeared to be multinucleate, extra large, or had a pycnotic, degenerate or odd-shaped nucleus.

Leydig cells were quantified according to the method of Heller, Lalli, Pearson & Leach (1971). This method involves counting all Leydig cell and Sertoli cell nucleoli from a minimum of ten randomly selected areas ten 4-μm sections apart in the biopsy specimen. These counts are then expressed as the ratio:

$$\frac{\text{No. of Leydig cells}}{\text{No. of Sertoli cells}}$$

Plasma FSH, ICSH and testosterone were assayed from blood samples taken during the control, CA administration and post-administration periods. Plasma FSH was measured using the method of Odell, Parlow, Cargille & Ross (1968). Plasma ICSH was measured by the method of Odell, Ross & Rayford (1967). Results were reported as μg standard/100 ml (radioimmunoassay; LER-907-standard). Plasma testosterone was measured using the method of Murphy (1970) and reported in ng/100 ml.

Eight consecutive 12-hr (overnight) urine samples were collected during the control period, during the CA administration period and during the post-administration period. Urinary FSH, ICSH and testosterone determinations were performed on aliquots of the 8-day pooled specimens. Urine was stored below 5°C until the procedures were carried out.

Urinary gonadotrophins were extracted according to the kaolin-acetone method of Albert (1955). Follicle-stimulating hormone was determined by the Steelman & Pohley (1953) method. Interstitial cell-stimulating hormone was determined using the increase in ventral prostate weights of 21-day-old hypophysectomized rats (Greep, Van Dyke & Chow, 1941). Urinary FSH and ICSH

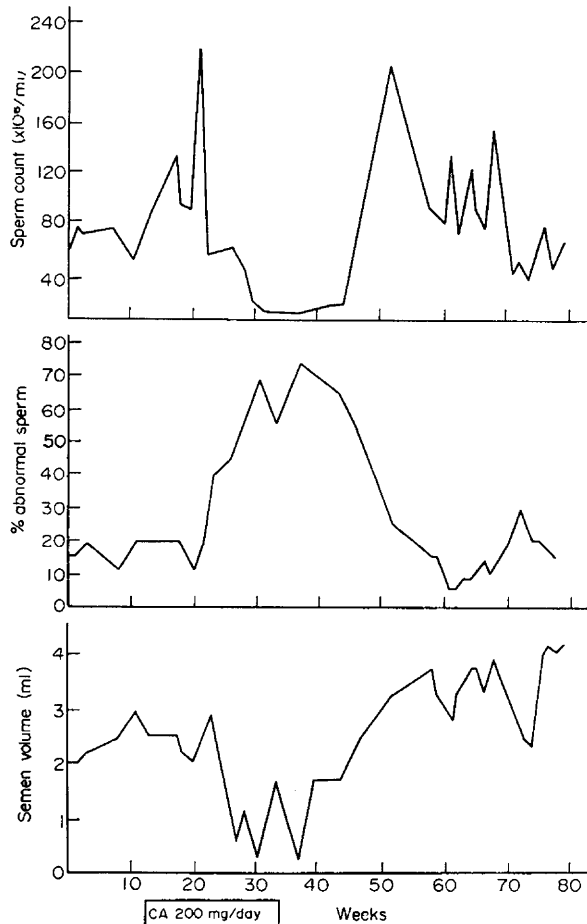
were expressed as mg/24 hr of a standard human urinary postmenopausal preparation (Pergonal P-26e). The urinary gonadotrophins were analysed statistically by the method of Thorslund & Paulsen (1963). Urinary testosterone and epitestosterone were extracted and analysed using a gas-liquid chromatographic method (Brusca, Kastella & Heller, 1966) and reported separately as $\mu\text{g}/24$ hr.

All results were analysed using Student's *t* test. For this report, $P \leq 0.05$ was the criterion used for statistical significance.

RESULTS

Effect on seminal fluid

Sperm concentrations changed with considerable uniformity among the six subjects and were similar to those shown in Text-fig. 1 for Subject 263. After



TEXT-FIG. 1. Sperm counts, % abnormal ejaculated spermatozoa and semen volume are shown with respect to control, CA administration and recovery periods (Subject 263). As the sperm count decreases, the % abnormal spermatozoa increases and the semen volume decreases.

beginning administration of CA, sperm counts fell below the control range within 5 to 12 weeks (average of 8 weeks) and reached functional sterility (below $20 \times 10^6/\text{ml}$) between 7 and 17 weeks (average of 13 weeks). After cessation of CA administration, sperm counts increased to within the control range by 8 to 12 weeks (average of 10 weeks), increased to a peak above control range by 12 to 16 weeks (average of 15 weeks), and returned to within control range in 17 to 27 weeks (average of 21 weeks). Motility decreased and later increased in consonance with the changes in sperm count. Five of the six subjects' sperm concentrations dropped to less than $1 \times 10^6/\text{ml}$, while two of these (Subjects 291 and 308) produced azoospermic ejaculates. The sixth subject (290) was probably also azoospermic, but was unable to submit specimens during this critical period due to temporary impotence.

In addition to a marked decrease in sperm concentration, the percentage of morphologically abnormal spermatozoa was greatly increased during CA administration (Text-fig. 1). In five out of six subjects (critical specimens unavailable for Subject 290), the percentage of abnormal spermatozoa increased from values averaging about 15% during the control period to about 80% during CA administration (100% abnormal spermatozoa was not uncommon). Therefore, the number of normal spermatozoa present in ejaculates during maximal suppression of spermatogenesis was exceedingly small. Recovery of sperm concentration was accompanied by a decrease in the percentage of abnormal spermatozoa.

Mean ejaculate volumes in control, administration and recovery periods for four men were 2.9, 1.5 and 2.6 ml, respectively, showing a reduction of about 50% followed by an essentially complete return to normal. The difference between the mean volumes of seminal fluid collected during periods of control and administration, is statistically significant (Student's *t* test, $P < 0.005$). There is no statistically significant difference between the control and recovery period volume ($P > 0.1$). Data from the other two men were deleted because few recovery samples were available. Text-figure 1 shows typical changes in seminal fluid volume during and after CA administration.

Effect on testicular histology

Data combined from all six subjects show that, during maximal effect from CA, the numbers of spermatogonia were reduced by 20%, spermatocytes by 35%, early spermatids by 71% and late spermatids by 82% (Table 1). Abnormal spermatocytes and spermatids increased with administration as follows: spermatocytes, from near zero to 40%; early spermatids from 1% to 7.5%; late spermatids, from 11% to 33%. No striking change in numbers of abnormal spermatogonia was found.

The three recovery biopsies available were taken 10 months (Subjects 263 and 308) and 6½ months (Subject 246) after cessation of CA administration. These biopsies, compared with corresponding control biopsies, generally showed control levels of spermatogonia, slightly reduced levels of spermatocytes and late spermatids, and slightly greater than control levels of early spermatids (Table 2). Both early and late spermatid numbers were markedly increased over administration values. The histogram of germ cell quantification for one

Table 1. Effects of cyproterone acetate on the number of cells of the germinal epithelium of human testes*

| Subject | Spermatogonia | Spermatocytes | Early spermatids (Sa—Sb) | Late spermatids (Sc—Sd) |
|---------------------|---------------|---------------|--------------------------|-------------------------|
| 246 Control | 1.66 | 1.90 | 1.57 | 1.83 |
| CA | 1.06 | 1.03 | 0 | 0.03 |
| % decrease† | 36 | 46 | 100 | 98 |
| 263 Control | 1.15 | 1.68 | 2.21 | 2.26 |
| CA | 1.02 | 1.21 | 0.60 | 0.50 |
| % decrease | 11 | 28 | 73 | 78 |
| 290 Control | 1.70 | 1.45 | 1.95 | 1.45 |
| CA | 1.48 | 1.36 | 1.19 | 0.45 |
| % decrease | 13 | 6 | 39 | 69 |
| 291 Control | 1.66 | 1.69 | 1.61 | 1.53 |
| CA | 1.22 | 0.65 | 0.005 | 0 |
| % decrease | 27 | 62 | ~100 | 100 |
| 296 Control | 2.17 | 3.96 | 4.60 | 3.10 |
| CA | 1.84 | 2.23 | 1.86 | 0.98 |
| % decrease | 15 | 44 | 60 | 68 |
| 308 Control | 1.74 | 2.00 | 1.78 | 1.61 |
| CA | 1.48 | 1.74 | 0.35 | 0.18 |
| % decrease | 15 | 13 | 80 | 89 |
| Average control | 1.68 | 2.11 | 2.28 | 1.96 |
| Average CA | 1.35 | 1.37 | 0.67 | 0.36 |
| Average % decrease‡ | 20 | 35 | 71 | 82 |

* Numbers expressed as the Sertoli cell ratio (number of a cell type/number of Sertoli cells).

† $\frac{\text{Experimental value} - \text{Control value}}{\text{Control value}} \times 100$.

‡ $\frac{\text{Average experimental value} - \text{Average control value}}{\text{Average control value}} \times 100$.

Table 2. Recovery of germinal cells in human testes after discontinuing administration of cyproterone acetate*

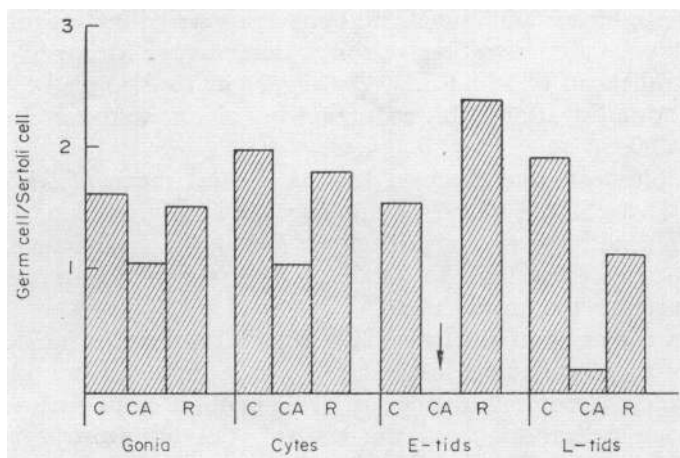
| Subject | Spermatogonia | Spermatocytes | Early spermatids (Sa—Sb) | Late spermatids (Sc—Sd) |
|-----------------------|---------------|---------------|--------------------------|-------------------------|
| 246 Control | 1.66 | 1.90 | 1.57 | 1.83 |
| Recovery | 1.51 | 1.68 | 2.30 | 1.10 |
| % difference† | ↓9 | ↓12 | ↑46 | ↓40 |
| 263 Control | 1.15 | 1.68 | 2.21 | 2.26 |
| Recovery | 1.17 | 1.33 | 1.82 | 1.06 |
| % difference | ↑2 | ↓21 | ↓18 | ↓53 |
| 308 Control | 1.74 | 2.00 | 1.78 | 1.61 |
| Recovery | 1.67 | 1.56 | 3.23 | 1.96 |
| % difference | ↓4 | ↓22 | ↑81 | ↑22 |
| Average control | 1.52 | 1.86 | 1.85 | 1.90 |
| Average recovery | 1.45 | 1.52 | 2.45 | 1.37 |
| Average % difference‡ | ↓5 | ↓18 | ↑32 | ↓28 |

* Numbers expressed as the Sertoli cell ratio (number of a cell type/number of Sertoli cells).

† $\frac{\text{Recovery value} - \text{Control value}}{\text{Control value}} \times 100$; ↑ = increase; ↓ = decrease.

‡ $\frac{\text{Average recovery value} - \text{Average control value}}{\text{Average control value}} \times 100$.

subject (246) for control, administration and recovery biopsies shown in Text-fig. 2 is typical for the whole group. The histological appearance of biopsies from these three periods are shown in Plate 1.



TEXT-FIG. 2. Sertoli cell ratios of spermatogonia (gonia), spermatocytes (cytes), early spermatids (E-tids) and late spermatids (L-tids) during control (C), cyproterone acetate administration (CA) and recovery (R) periods (Subject 246).

Subjective examination of the Leydig cells of all biopsies showed no obvious changes in morphology or number, although a slight ruffling of nuclear membranes was noted in Leydig cells in three out of six of the administration biopsies. Control and administration biopsies for Subject 246 were quantified with the following results: control $0.15 \pm \text{S.D. } 0.05$, administration $0.16 \pm \text{S.D. } 0.03$. Evaluation with Student's *t* test indicated that no significant change in Leydig cell number occurred ($P > 0.3$).

Effect on plasma hormone levels

Plasma testosterone was measured in each of the six subjects. Their mean control value and standard deviation was 556.3 ± 157.1 ng/100 ml. During CA administration, there was a significant drop in plasma testosterone to 329.8 ± 194.6 ng/100 ml ($P = 0.05$). Postadministration values returned to control levels (548 ± 170.5 ng/100 ml).

Plasma FSH was measured in each of the six subjects. Their control mean was $32.6 \pm \text{S.D. } 3.1$ μg standard/100 ml (radioimmunoassay; LER-907-standard[†]). No statistically significant difference ($P \leq 0.05$) was found between the control values, the CA administration values ($32.9 \pm \text{S.D. } 4.1$ μg LER-907/100 ml) and postadministration values ($32.3 \pm \text{S.D. } 0.9$ μg LER-907/100 ml). (Normal control range, 22.0 to 45.3 μg LER-907/100 ml; mean = $34.2 \pm \text{S.D. } 8.6$ μg LER-907/100 ml.)

Plasma ICSH was measured in each of the six subjects. Their control mean was $7.6 \pm \text{S.D. } 2.2$ μg LER-907/100 ml. No statistically significant difference ($P \leq 0.05$) was found between CA administration values ($7.5 \pm \text{S.D. } 3.4$ μg LER-907/100 ml) and postadministration values ($7.9 \pm \text{S.D. } 1.9$ μg LER-907/

100 ml). (Normal control range, 4.7 to 15.3 μg LER-907/100 ml; mean = 9.0 \pm S.D. 2.6 μg LER-907/100 ml).

Effect on urinary hormone excretion

Urinary testosterone and epitestosterone were assessed in four subjects during control and CA administration. Urinary testosterone dropped significantly from a control mean of 73.8 \pm S.D. 20.8 $\mu\text{g}/24$ hr to 21.5 \pm S.D. 8.1 $\mu\text{g}/24$ hr during CA administration. This reduction in urinary testosterone was significant at $P = 0.005$.

Urinary epitestosterone dropped from a control mean of 54.5 \pm S.D. 32.6 $\mu\text{g}/24$ hr to 11.8 \pm S.D. 4.5 $\mu\text{g}/24$ hr during CA administration. This decrease in urinary epitestosterone was significant at $P = 0.025$. Insufficient postadministration values were available to obtain valid statistical results; however, they appeared to return to control levels.

Data from the assay of FSH and ICSH in thirty-seven urine collections, in which two or more unknown dosage levels were obtained, were tested using an IBM 7040 and the computer program of Thorslund & Paulsen (1963). Data included urine collections from the control, CA administration and postadministration periods. The F ratios of variation between preparations and due to parallelism and linearity were used to determine the acceptability of the assay. The lambdas (λ) of the assays ranged from 0.073 to 0.124 with a mean of 0.094. All other values were obtained from the interpolation tables.

Measuring urinary ICSH revealed no significant difference between control values (0.91 \pm S.D. 0.44 mg P-26e/24 hr) and CA administration values (0.72 \pm S.D. 0.51 mg P-26e/24 hr) ($P \leq 0.05$). Insufficient postadministration values were available to obtain valid statistical results. With those values obtained, however, there appeared subjectively to be no difference as they fell within the subjects' control ranges.

Urinary FSH values for both control and administration periods were quite variable, but detectable in all samples. The values all fell within the normal control range for our laboratory (0.02 to 0.22 mg P-26e/24 hr).

Clinical observations

Tests for liver or kidney function during CA administration revealed normal values. However, the pH of urine in four subjects changed from slightly acidic to neutral and two of these subjects reported two to three episodes of nocturia. Of the four subjects in whom blood cytology was performed, all showed a slight increase in lymphocytes but no other irregularities. An increase in the white

EXPLANATION OF PLATE 1

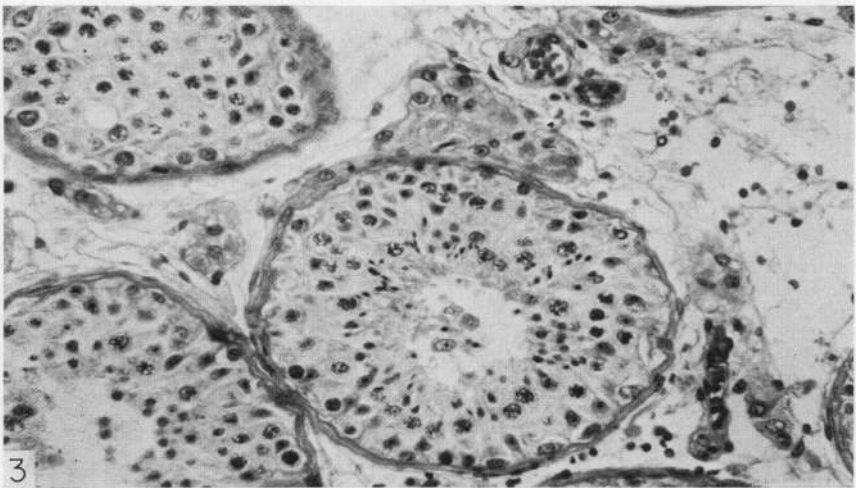
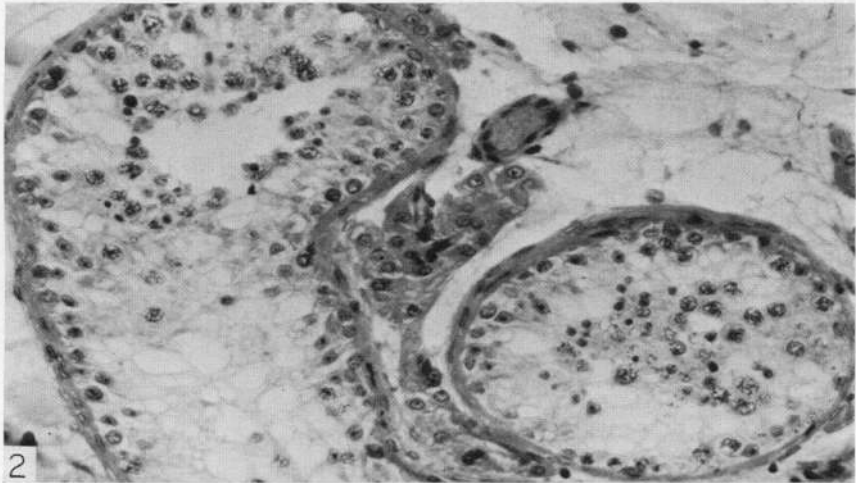
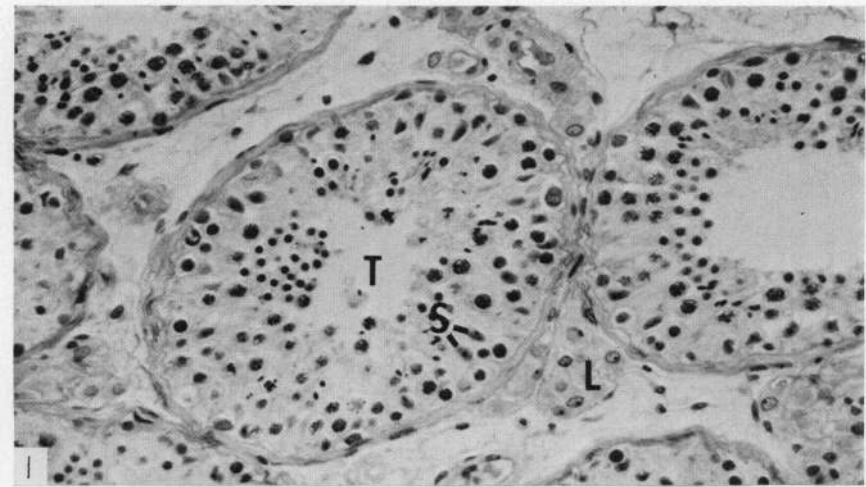
Photomicrographs of sections of testicular biopsies taken at three different times (Subject 263). Cleland's fixative, Fe-H and E stain. Seminiferous tubule (T); Leydig cells (L); Sertoli cell nucleus (S). $\times 280$.

FIG. 1. Preadministration biopsy.

FIG. 2. Biopsy after 3½ months on CA. Seminiferous epithelium is reduced to Sertoli cells and a few germ cells. Many spermatocytes and early spermatids are sloughed into the lumen. Leydig cells appear to be normal.

FIG. 3. Recovery biopsy taken 10 months after cessation of CA. Germinal epithelium and Leydig cells appear to be normal.

PLATE 1



(Facing p. 372)

blood cell content of the semen was observed in four out of six of the subjects.

Information about effects on libido and potency was obtained through repeated confidential discussions with the subjects. Reduction of libido was reported within 1 to 4 weeks and was completely lost within 10 to 14 weeks following the beginning of administration. Potency was reduced in all subjects and almost completely lost in four within 10 to 14 weeks after starting CA. Difficulties in achieving orgasm during maximum CA effect are well attested to by the paucity of seminal fluid samples submitted during this period. After administration ceased, libido and potency were reported to reappear in 1 to 2 weeks (one subject reported an increase in 1 day) and were up to control levels in 2 to 4 weeks.

The subjects denied any changes in personality or emotions. One subject lost about 10 lb, two gained 10 to 15 lb, while three remained steady during treatment. The single instance of weight loss was associated with cessation of strenuous exercise. After CA was discontinued, gynaecomastia of the small, plaque-like form was found in two subjects. One (263) reported some tenderness and swelling on both sides 1 week after administration ceased, while the other (291) had slight gynaecomastia on one side 4 months after CA was stopped.

DISCUSSION

Data from the six male subjects cooperating in this study show that 200 mg CA/day causes a moderate decrease in semen volume, testicular germ cell number, and plasma and urinary testosterone levels and a dramatic decrease in sperm count, libido and potency. No significant change was seen in plasma or urinary ICSH and FSH levels or in Leydig cell morphology or numbers.

The striking decrease in germ cell numbers in the germinal epithelium of the testis during CA administration indicates that the major site of inhibition is in the testis rather than in the epididymis or remaining ductular system. Germ cell numbers were reduced most strikingly as they became more mature. Defects incurred in spermatogonia might cause death immediately or later in development as they become spermatocytes or spermatids. It is of interest that this pattern of depletion is similar to that caused by testosterone enanthate administration (Heller, Morse, Su & Rowley, 1970). Possible causes for this reduction in spermatogenesis will be discussed later.

In view of the reduction of all major germ cell types, one would also expect an increase in FSH, as occurs, for example, after depletion of germ cells by radiation (C. G. Heller, unpublished data). The fact that such an increase was not detected is reminiscent of the failure of FSH to increase during germ cell depletion caused by testosterone treatment (Heller *et al.*, 1970). It is possible that the considerable numbers of spermatogonia and spermatocytes remaining during CA administration (85% and 65% of control, respectively) may be sufficient to keep FSH levels in the control range. On the other hand, the progestational activity of CA may be balancing the tendency for increase in FSH by partial central inhibition. Schoonees, Schalch & Murphy (1971) have reported a transient decrease in plasma FSH in men suffering from prostatic carcinoma who were given 200 mg CA/day, while Vosbeck & Keller (1971),

using the same dose, have shown a reduction in urinary FSH in some of their normal male subjects.

The lack of change in numbers or morphology in Leydig cells is consonant with our finding that ICSH levels remain unchanged during CA administration. There have been reports of changes in human Leydig cells (Markewitz *et al.*, 1969; Stadler, Horn & Moormann, 1971), but the lack of change in ICSH has been commonly found (Geller, Baron, Warburton & Loh, 1969; Schoonees *et al.*, 1971; Walsh, Swerdloff & Odell, 1971). In spite of this lack of change in ICSH, we have detected a decrease in plasma and urinary testosterone during CA administration. This finding has been confirmed by several other workers (Scott & Schirmer, 1966; Geller, Fruchtman, Newman, Roberts & Silva, 1967; Geller *et al.*, 1968; Scott & Wade, 1969; Geller *et al.*, 1969; Schoonees *et al.*, 1971).

The possibility exists that CA could cause a change in plasma testosterone level by a change in kidney function or a reduction in binding by testosterone-binding globulin causing an increase in metabolic clearance rate. This is unlikely in view of the fact that a decrease in urinary testosterone was also detected in this study and by Geller *et al.* (1969). In addition, they also found that injected testosterone propionate was excreted by men in the same quantities with or without simultaneous CA administration, and that CA inhibited the increase in testosterone excretion stimulated by HCG. In view of this evidence, we concluded that CA may be causing an inhibition of testosterone secretion, either directly or by some indirect means other than ICSH reduction.

It has been demonstrated that cyproterone (free alcohol form) can inhibit testosterone synthesis in rat testis microsomes (Breuer & Hoffman, 1968) but this has not been shown in the human or for the distinctly different CA (acetate form). Cyproterone in the free alcohol form also presents the enigma of causing an increase in testosterone levels in men without causing any significant increase in total gonadotrophins (Apostolakis, Tamm & Voigt, 1968; Rausch-Stroomann, Petry, Hocevar, Mauss & Senge, 1970). Clarification of the direct effects of cyproterone (free alcohol form) and CA on human Leydig cell function await studies on testosterone synthesis *in vitro* by testicular biopsies.

The reduction in libido, potency, sperm concentration and semen volume (a measure of accessory organ function) could all be caused by androgen deprivation. The major cause of deprivation might be brought about by a competition between CA and androgens for binding-sites in androgen-dependent tissues. This effect would be increased by the concomitant reduction in testosterone level. It could be conjectured, however, that the reduced testosterone level alone might be sufficient to account for these changes without any antiandrogenic effect. Data are lacking to show the level of testosterone which is sufficient to maintain masculinization in normal men.

The germinal epithelium is known to require both testosterone and FSH to carry out normal spermatogenesis (Heller, Lalli & Rowley, 1968). With no change in FSH, the lack must be presumed to concern the availability of testosterone or possibly a direct 'poisoning' effect from CA or its metabolites. Such an effect has been suggested for progesterone (Heller, Moore, Paulsen, Nelson & Laidlaw, 1959) and for clomiphene citrate (Heller & Heller, 1970). The

demonstrated effect of CA on the germinal epithelium does not rule out the possibility that androgen deprivation, possibly in the epididymis, could inhibit sperm maturation and thereby further reduce sperm concentration in the seminal fluid.

An antiandrogen has been defined as 'a blocking agent of androgen receptors' (Neumann *et al.*, 1967). This term is not meant to include agents which induce testicular atrophy and/or reduction of testosterone production primarily by virtue of their ability to inhibit gonadotrophin secretion or by directly inhibiting testosterone synthesis. It is possible, however, that antiandrogen activity could cause a reduction in testosterone synthesis by enzyme inhibition.

Several investigators have shown in the rat that CA, acting either *in vivo* or *in vitro*, reduces the ability of androgen-dependent tissues to bind radioactivity from [³H]testosterone (Woolman & Hamilton, 1968; Whalen & Luttage, 1969; Stern & Eisenfeld, 1969; Geller *et al.*, 1969; Fang & Liao, 1969; Walsh & Korenman, 1970). In man, however, few experiments have been carried out to elucidate the mode of CA action on target organs. Jonsson (1969) incubated slices of human hypertrophied prostate and [³H]testosterone, with and without CA. Slices which were then simply blotted, digested and counted showed no change in ³H uptake with the addition of CA. However, if the slices were washed for 1 hr with steroid-free medium, there was a suggestion of a reduction in retained ³H in slices incubated with CA.

Hansson & Tveter (1971), using benign nodular hyperplastic prostate, but employing cyproterone (free alcohol form), showed a decrease in androgen-binding by slices, as well as cytosol or nuclear fractions. This again emphasizes the importance of differentiating between the free alcohol form of cyproterone and CA. As yet, there appears to be no firm evidence that CA is indeed competing with androgens for target-organ-binding sites (i.e. that CA is acting as an antiandrogen) in human tissue. In spite of this lack of direct evidence, it seems most likely that the observed decline in libido, potency, sperm concentration and seminal fluid volume are caused by a combined effect of the reduction in circulating testosterone and a competitive inhibition for receptor sites by CA.

The striking reduction in sperm concentration in ejaculates which were also reduced in volume indicates that CA is indeed a powerful antifertility agent in normal men. At the dose we employed, the loss of libido and potency was severe, although some of the subjects were able to supply semen specimens throughout the entire administration period. It is possible that this undesirable effect may be abolished by use of a smaller dosage which may still inhibit spermatogenesis. In this event, CA could be a candidate for use as a male contraceptive.

ACKNOWLEDGMENTS

We wish to thank Dr Martin Friedrichs, of Berlin Laboratories, New York, an affiliate of Schering AG, Berlin, Germany, for kindly supplying the cyproterone acetate. We thank Mrs Carole Marty, Mr Mike Su and Mr Neil Horike for their technical assistance.

This investigation was supported by funds from The Ford Foundation (No. 68-0806) and The Population Council (No. M67.122), and was presented in part at the 53rd Annual Meeting of The Endocrine Society, San Francisco, California, 24th to 26th June 1971.

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