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EFFECT OF CYPROTERONE ACETATE ON CLINICAL, ENDOCRINE AND PATHOLOGICAL FEATURES OF BENIGN PROSTATIC HYPERTROPHY

JACK GELLER,* JACK FISHMAN† and THOMAS L. CANTOR‡

*Mercy Hospital and Medical Center, 4077 Fifth Avenue, San Diego, California 92103, U.S.A.
†Institute for Steroid Research, Montefiore Hospital, 210th and Bainbridge Avenue, Bronx, New York 10467, U.S.A.
‡Mercy Hospital and Medical Center, 4077 Fifth Avenue, San Diego, California 92103, U.S.A.

SUMMARY

The absence of BPH in castrated or hypopituitary patients suggests that the disease is related to the amounts of secreted sex steroids—testosterone (T) and estrogen (E)—and perhaps to either GH or prolactin. We have, therefore, attempted medical castration with progestational anti-androgens. This study reports the objective effects of a progestational anti-androgen, cyproterone acetate (CPA), on the clinical symptoms of BPH and correlates these changes with the effects of the drug on sex steroid production and the action of androgens at the prostate cellular level. CPA was administered prior to TURP. Significant decreases occurred in plasma T, urinary TG, and E production rates, but not in plasma LH. CPA also decreased LH and HGH reserves. *In vitro* incubation of CPA with prostate minces from controls decreased binding of H^3-DHT to prostate cytosol receptors. Histologic studies of prostate tissue from patients treated with CPA indicated atrophy and embryonal changes of glandular elements. Three of 4 patients treated with CPA voided spontaneously within 2 months of therapy. In summary, CPA decreased sex steroid synthesis by the testes without suppression of LH. CPA also appeared to block steroid–protein binding in the prostate. These hormonal and biochemical effects correlated with clinical improvement indicated by the ability to void in patients in retention prior to therapy.

Although the pathogenesis of benign prostatic hypertrophy (BPH) is not understood, the absence of the disease in castrated or hypopituitary patients strongly suggests that BPH is related to either an abnormality in sex steroid secretion by the testis or to an abnormal amount of testosterone or estrogen, or their metabolites, within the prostate. Further support for the importance of testicular steroids in the pathogenesis of BPH comes from previous reports by Huggins[1], Cabot[2], and White[3], that castration decreased prostate size and symptoms of prostatism in patients with established BPH.

It seemed reasonable, therefore, to study in patients with BPH the effect of a progestational anti-androgen, 1,2α-methylene-6-chloro-pregna-4,6-dien-17α-ol-3,20-dione-17-acetate, cyproterone acetate (CPA), which blocks both the secretion of the testis sex steroids and the effects of androgen at the cell level [4]. This study, therefore, reports the objective effects of CPA on the clinical symptoms of BPH and also correlates these changes with the effects of the drug on prostate pathology, sex steroid production, LH secretion and reserve, and the biochemical action of androgens at the prostate cellular level. Since growth hormone (GH) has been implicated in the regulation of prostate growth [5], the effects of CPA on GH reserve were also studied.

Reprint requests to: Jack Geller, M.D., Mercy Hospital and Medical Center, San Diego, California 92103, U.S.A.

METHODS

Clinical

Five patients with advanced BPH, including four in urinary retention with catheter drainage, were selected for therapy with CPA, 250 mg daily by mouth, for two-to-four months prior to open prostatectomy. Patients served as their own controls and were maintained on other medications required for concurrent chronic illnesses throughout this study. They were hospitalized on a metabolic ward prior to and at intervals following treatment, for evaluation of clinical status and endocrine function. Complete endocrine function studies as listed below were not necessarily done for each patient. In some instances, patients were studied with only one test while others were submitted to the entire battery of endocrine studies. In order to increase the amount of available data, endocrine data obtained from several patients with carcinoma of the prostate, treated with 250 mg daily of CPA, has been added to some of the studies of patients with BPH.

Laboratory

Plasma testosterone was measured by Biomedical Assay Laboratories Division of New England Nuclear Corporation, using a double-isotope derivative technique [6]. Coefficient of variation with this technique is 5·7%. Testosterone glucuronide (TG), as an index of testosterone production, was measured in our

laboratory by a modification of the method of Futterweit *et al.*[7] by thin-layer chromatography and gas–liquid chromatography. Error of this method is ±3%; sensitivity to 5 μg/24 h. In this modification, the trimethylsilyl (TMS) ether derivatives are formed from urine extracts purified on thin-layer chromatography. The TMS ethers are then rechromatographed on thin layer plates of silica gel G for final purification and assay with gas–liquid chromatography using 4′-SE-30 columns. 1-Dehydrotestosterone was used as an internal standard for quantitation with gas chromatography. Plasma LH and GH were measured by the radioimmunosorbent technique of Wide[8]. Sensitivity of the LH method was to 3 mIU/ml of the second IRP; sensitivity of the HGH assay was to 0·5 ng/ml. The per cent standard error of both assays was ±5%. Estrogen production was measured by the isotope dilution technique following injection of H^3-estradiol and measurement of the specific activity of urinary estrone and estriol [9]. Pituitary LH reserve was assessed by measurement of changes in plasma LH following clomiphene administration, 200 mg daily, according to the method of Bardin *et al.*[10]. Tests for GH reserve were performed according to the technique of Merimee *et al.*[11], following a priming dose of 10 mg daily of diethylstilbestrol for two days. Leydig cell reserve was measured by studying changes in TG excretion on the fourth day of administration of chorionic gonadotropin, 2500 U per day.

Average acid phosphatase content of the prostate was determined from four separate aliquots of prostate that were homogenized and assayed for enzyme content within four hours. Acid phosphatase activity of the prostate was measured using *p*-nitrophenyl phosphate substrate and 0·09 M citrate buffer, pH 4·8. 0·2 ml of suitably-diluted aqueous tissue homogenates, plus buffer and substrate, were heated for 30 min at 37°C. Sodium hydroxide, 0·1 M, was then added and color intensity of unknowns was read at 410 nm in a Beckman DU spectrophotometer. Values were expressed as sigma units obtained by comparison to a standard curve.

The effect of CPA on steroid–protein binding in the human prostate was studied in minces of human prostate obtained at surgery. Tissue was weighed and pre-incubated for 30 min with either ethanol alone or CPA, 10^{-6} M in Eagle's MEM at 37°C. [1,2,6,7-H^3]-testosterone was then added for an additional 1½ h incubation. All operations subsequent to this point were carried out at 0–4°C. The tissue was washed by centrifugation with Medium A (0·32 M sucrose; 1·0 mM magnesium chloride; 0·02 Tris–HCl, pH 7·4) and homogenized with a Potter–Elvehjem Teflon homogenizer; the cytosol fraction was obtained by ultracentrifugation at 105,000 ***g*** for 1 h. The cytosol was fractioned into 1·5 ml aliquots on a water-jacketed column consisting of 7 cm. of G-50 Sephadex on top of 15·5 cm. of Bio-Gel A 1·5 M. Aliquots of each eluted fraction were counted for isotope in Liquifluor scintillator in a Beckman LS-133 liquid scintillation counter and assayed for protein by the method of Lowry. Radioactive peaks were quantitated as c.p.m./mg protein. The effect of CPA on the peak representing the receptor protein has been studied.

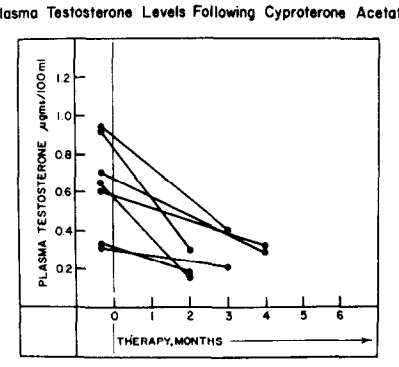

Fig. 1. This figure depicts changes in plasma testosterone levels following therapy with CPA. Each dot to the left of the zero indicates individual control values of plasma testosterone prior to therapy. A solid line connects the control value to the post-treatment value.

RESULTS

1. The effect of CPA on plasma testosterone (Fig. 1). Plasma testosterone in seven patients prior to therapy averaged 0·6 μg per 100 ml. Two-to-four months following treatment, values decreased in each patient to an average of 0·35 μg per 100 ml. Greater decreases occurred in patients with higher control values.

2. The effect of CPA on urinary TG (Fig. 2). The average value for urine TG in seven patients prior to treatment was 30 μg/24 h (normal 30–200). Following one-to-eight weeks of therapy, values fell in each patient, and the average for the group decreased to 15 μg/24 h.

3. The effect of CPA on recovery of urinary TG was studied in two patients (Fig. 3). It could be seen that the percentage recovery of injected testosterone, 25 mg I.M., as TG was similar before and following CPA.

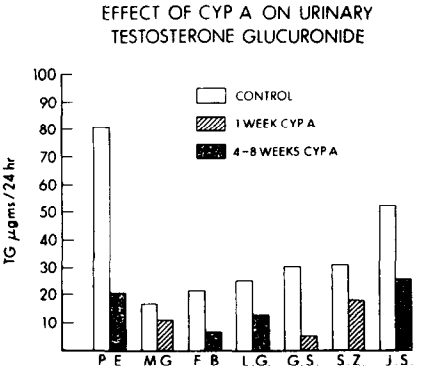

Fig. 2. This figure depicts changes in urinary testosterone glucuronide following treatment with CPA. Patient initials are shown beneath each set of values.

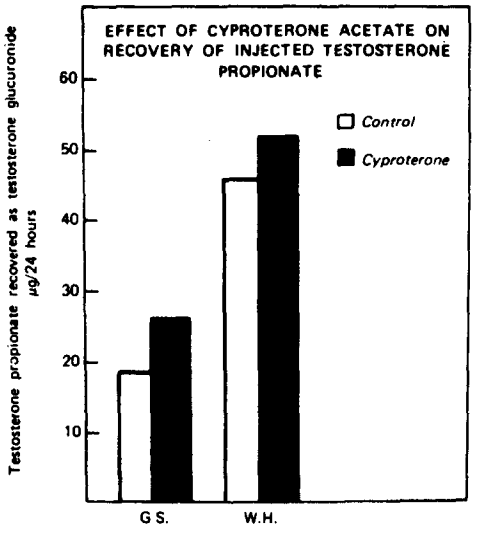

Fig. 3. This graph depicts the recovery above baseline values of urinary testosterone glucuronide following injection of testosterone propionate, 25 mg I.M., before and after CPA treatment in two patients.

4. The effect of CPA on estrogen production rates (Table 1). Approximately two months following treatment with CPA, the production rate of estradiol in patients W.H. and M.D., as calculated from specific activity of urinary estrone or estriol, decreased by approximately one-half. The treatment altered the metabolism of estradiol, in that less estrone, 2-hydroxyestrone and 2-methyoxyestrone, but more estriol, was formed.

5. Clinical effects of CPA in patients with BPH. In five patients treated with CPA, 250 mg daily by mouth for two-to-four months, all improved clinically (Table 2) by both objective (residual urine and ability to void) and subjective criteria. The resected prostate was surprisingly small compared to the expected values estimated prior to drug treatment. In a previous study of ten control patients treated with catheter drainage alone but without other specific therapy, only three out of ten were able to void spontaneously after two or more months [12].

TABLE 1

PRODUCTION RATES OF ESTRADIOL AS CALCULATED FROM ESTRONE AND ESTRIOL

Subject	W. H.		M. D.	
	Before	After	Before	After
Estrone	109	59	136	76
Estriol	117	80	107	61

All figures are in µg per day.

6. Effects of CPA on prostate histology. Appreciable differences between CPA-treated patients and control patients with untreated BPH are summarized in Fig. 4. Most, but not all, features are readily appreciable. These differences between treated and untreated patients include the following: the epithelial cells in treated specimens tend to be more cuboidal in many areas in lieu of the tall columnar cells reported by others. In sections from CPA-treated patients, there seemed to be fewer nodules in the same comparable area of the prostate. In general, nodules are smaller and contain fewer and less distended acini. In addition, smaller glandular acini are seen. Another difference is the evident loss or only minimal papillation of lining glandular epithelium in the treated patients, even in areas of tall columnar cells. The cells show midzonal positioning of nuclei in contrast to the more basal position of the untreated group. The basal cells are a bit more prominent in the section from a treated patient. Some embryonal changes in the glandular elements of the prostate are noted in the CPA-treated group.

7. Prostatic acid phosphatase changes following CPA treatment. Acid phosphatase content of the resected human prostate was consistently low in CPA-treated patients as compared to controls (Fig. 5). Although overlap of individual values is present in

TABLE 2

Patient	* Months of Therapy	Symptoms of Prostatism		Estimated Size of Prostate		Prostate Size At Surgery
		Before Therapy	After Therapy	Before	After	
F. B.	2 1/2	Foley Catheter	Spontaneous voiding Residual = 20 ml.	3+	1+	27 gm.
L. G.	2 1/4	Foley Catheter	Spontaneous voiding Residual = 300 ml.	2+	1+	14 gm.
P. E.	4	4+	3+	2+	1+	12 gm.
W. H.	3	Foley Catheter	Foley Catheter	2+	1+	20 gm.
M. D.	3	Foley Catheter	Spontaneous voiding Residual = 40 ml.	3+	1+	Operation deferred

* Drug and Dosage: Cyp A, 250 mg/day.

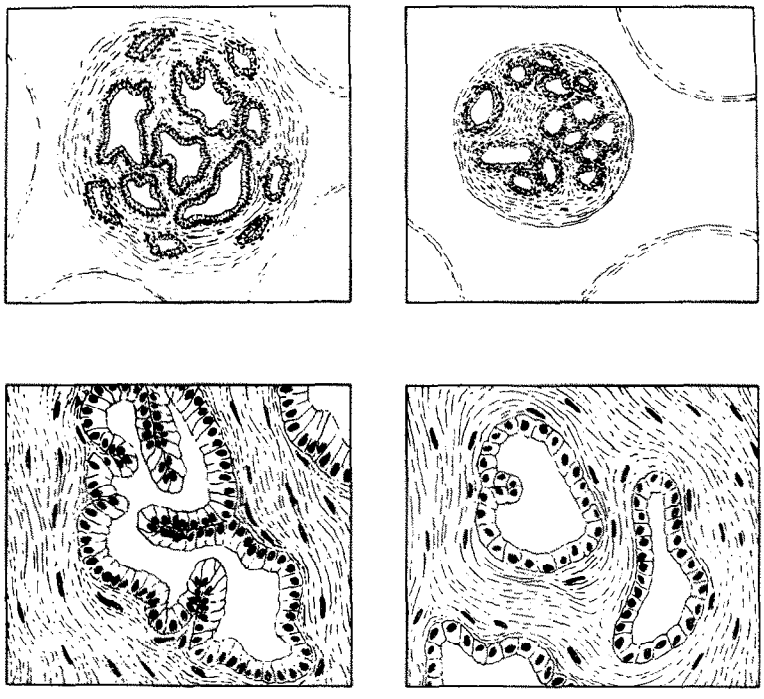

Fig. 4. Composite diagram of histologic changes in prostate during progestational hormone therapy. *Top*, In treated group (right), smaller nodular areas and smaller glandular acini are noted than in those of untreated group (left). *Bottom*, Glandular epithelium in untreated group (left) shows tall columnar epithelial cells with basal nuclei and abundant papillary projections; treated group (right) shows cuboidal to columnar epithelial cells with basal to midzonal nuclei, less and inconspicuous papillation.

the two groups, averages are well separated. This finding is supported by a decreased standing for acid phosphatase histochemically in the prostates of CPA-treated patients.

8. The effect of CPA on basal LH secretion (Fig. 6). For each of the patients in whom TG was measured before and following CPA, plasma and urine LH were also measured. Note that little or no change from control levels occurred in these values during drug administration.

9. The effect of CPA on Leydig cell reserve (Fig. 7). Urinary TG on the fourth day of HCG stimulation is shown for six patients before and during CPA therapy. Note that in every instance, except patient H.P. whose control values indicated little if any Leydig cell function, there was a notable reduction in urinary TG on the fourth day of HCG stimulation during CPA therapy as compared to the control responses.

10. The effect of CPA on LH reserve (Fig. 8).

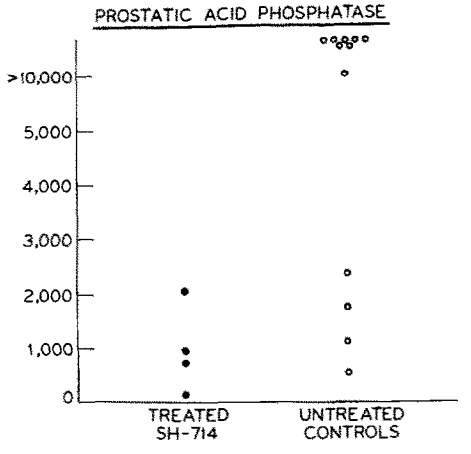

Fig. 5. This figure shows the content of prostatic acid phosphatase in resected human prostates in patients treated with cyproterone acetate (CPA) and in untreated controls. Values in sigma units per gram are shown along the vertical axis. Each dot represents the average of four aliquots of the same prostate gland.

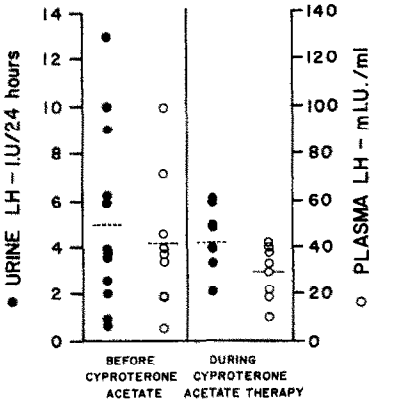

Fig. 6. This figure compares the values for LH in urine and plasma before and two-to-four months following CPA therapy.

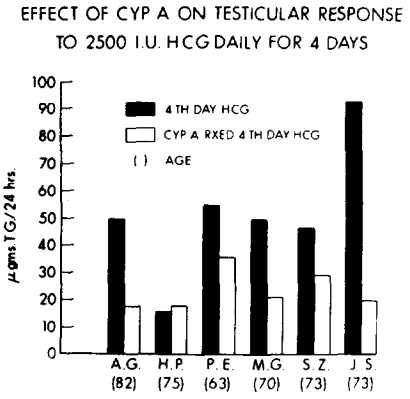

Fig. 7. This figure depicts the effect of CPA on a testicular response to human chorionic gonadotropin given daily for four days.

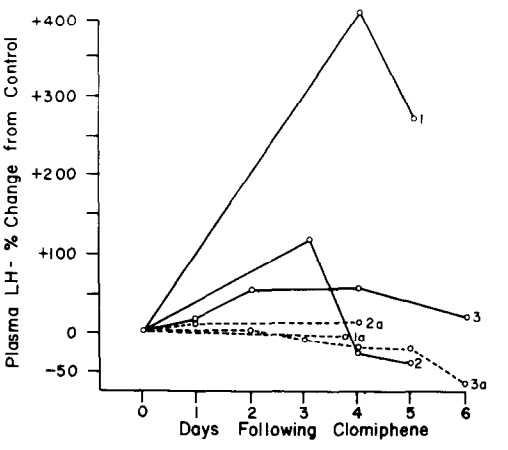

Fig. 8. This figure depicts changes in plasma LH as a per cent change from control values in plasma LH following clomiphene therapy for six days in three untreated patients (solid lines) and in the same patients during treatment with CPA (dotted lines). Control values of LH ranged from 5–52 m.i.u./ml of second IRP standard.

Three patients showed partial responses to clomiphene, as indicated by transient rises in plasma LH during a six-day clomiphene test. Repeat study during CPA administration resulted in a complete blunting of the LH response.

11. The effect of CPA on GH reserve (Fig. 9).[1] Two patients with normal responses of plasma GH following arginine stimulation prior to CPA therapy were restudied at one month and two months following the start of drug treatment. In patient W.H., the response one month after start of therapy was blunted with a rise to less than 5 ng per ml. However, at two months, the GH rise in plasma following arginine stimulation was perfectly normal and paralleled the original response. In patient M.D., responses to arginine, as indicated by the rise in plasma GH, were blunted at both one month and two months, with a rise to less than 5 ng/ml occurring as the maximum response.

12. The effects of CPA on steroid-protein binding in human prostate. Pre-incubation of human prostate minces with 10^{-6}-CPA significantly decreased the binding of H^3-steroid to receptor protein represented by the second elution peak of fractionated human prostate cytosol (Fig. 10).

DISCUSSION

Progestational agents have been previously demonstrated to be effective in therapy of carcinoma of the prostate and BPH [12, 13, 14]. In this report of five patients with BPH, three out of four in urinary retention were able to void spontaneously after CPA for two-to-four months. The fifth showed a decrease

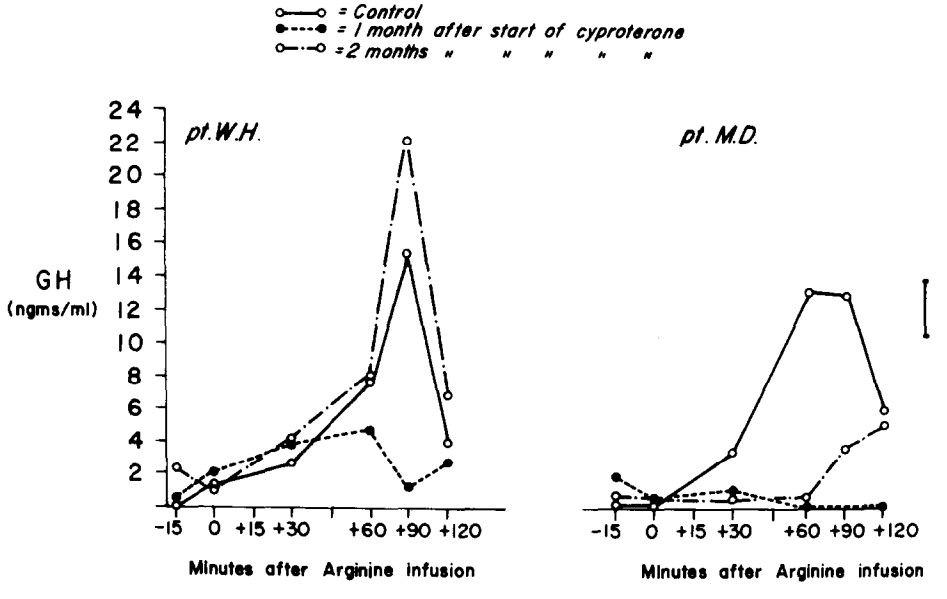

Fig. 9. This figure shows the effect of CPA on GH changes following an arginine infusion.

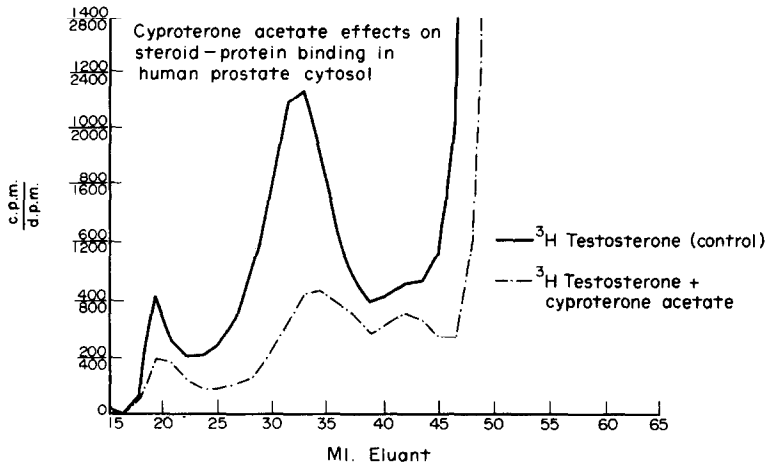

Fig. 10. Fractionation on G-50—Bio-Gel A 1·5 M duo-column of cytosol derived from prostate minces pre-incubated with and without CPA, 10^{-6} M. Note marked decrease in second steroid–protein peak in presence of CPA.

in residual urine and improvement in symptoms of prostatism. While the number of studies with CPA is small, these favorable clinical results are accompanied by evidence in almost each case of decreased prostate size and activity. Histologic changes after CPA, including decreased size of acini and embryonal changes, along with decreased content of acid phosphatase, suggest prostate atrophy.

The precise mechanism by which CPA produces such atrophy is not yet clear, since we have demonstrated multiple effects on the endocrine system of 250 mg daily of CPA, including suppression of testosterone secretion, estrogen production, LH reserve, and GH reserve. No effects have been noted on adrenocortical function [15]. The decrease in plasma testosterone and urine TG appears to be a direct effect on the testes, as indicated by the lack of change in plasma and urine LH and the inhibition of Leydig cell response to HCG noted during CPA therapy. The absence of effect on CPA on recovery of injected testosterone (Fig. 3) is against the possibility that a change in metabolic clearance rate or change in pathway of testosterone metabolism may have decreased plasma testosterone and urine TG levels. There is conflicting evidence in the literature on the effect of progesterone and synthetic progestational agents on pituitary gonadotropin release, particularly LH. A positive feedback effect, with an increase in LH, has been demonstrated by Taleisnik *et al.*[16]. Loraine *et al.*[17], has shown no change in plasma or urine LH, but inhibition of the mid-cycle plasma LH peak when synthetic progestational agents were used for birth control. Provera has been shown by Rifkin *et al.*[18], to decrease plasma LH. Our data strongly suggest that CPA does not suppress LH. However, the expected rise in LH following a decrease in plasma testosterone does not occur, indicating an interference with the feedback regulation of LH. This has been confirmed by demonstrating that pituitary LH release following clomiphene is blunted or completely prevented by CPA (Fig. 8).

We have demonstrated a variable effect of CPA in doses used (250 mg/day) on GH release following arginine. In one patient (M.D.), absence of GH response was demonstrated at one and two months following the start of CPA after a normal control study. W.H. showed a blunted response at one month, but a normal response three months following the institution of CPA therapy.

Simon *et al.*[19], demonstrated inhibition of GH release following acute administration of Provera. This recently has been confirmed by Lawrence and Kirsteins[20] in acromegalics. The relationship between GH and prostate tumors is still indirect.

The decrease in estrogen to the castration levels demonstrated in two patients during CPA therapy may reflect either a decrease in estrogen secondary to a decrease in testosterone which serves as a precursor for the estrogen, or it may reflect the direct inhibition of estrogen synthesis by Leydig cells. Since estrogen may stimulate squamous metaplasia and fibromuscular growth in the middle and lateral lobes of the prostate, the usual anatomic sites of BPH, decreased estrogen production rates with CPA may have relevance to the clinical effects of the drug in BPH.

It is, of course, possible that the demonstrated effects of CPA to compete at the prostate cellular level for cytosol dihydrotestosterone receptor sites, as shown in this study (Fig. 10), may be equally or more important in controlling prostate tumor growth than changes in extracellular hormone concentrations.

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