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Testosterone Metabolism in Patients with Advanced Carcinoma of the Prostate: A Comparative in Vivo Study of the Effect of Oestrogen and Anti-Prolactin

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Summary. In the light of the high incidence of cardiovascular side effects with oestrogen therapy in patients with prostatic cancer, other medications altering androgen metabolism are under investigation. The influence of the anti-prolactin bromocriptine (CB154) on plasma kinetics of testosterone and on endogenous hormones was studied and compared with the effect of ethinyl oestradiol in 25 patients with prostatic carcinoma. Bromocriptine significantly suppressed both prolactin and testosterone, inhibited the transfer of androgen from the inner pool into the deep compartment and favoured its degradation. Ethinyl oestradiol decreased testosterone, LH and FSH, and prolonged the biological half-life of testosterone. The effects of bromocriptine on androgen metabolism might be of therapeutic value in patients with prostatic carcinoma.

Key words: Prostatic carcinoma - Plasma kinetics - Testosterone - Ethinyl oestradiol - Anti-prolactin Bromocriptine.

As many as 45-80% of patients with advanced cancer of the prostate have metastases when first seen by a physician (8, 16, 28) and therefore would require treatment that is based mainly on oestrogen preparations. However it has been said that "what oestrogen treatment wins from the cancer, it more than loses to other causes of death" (25, 26, 27). This pessimistic statement by the Veterans Administration Cooperative Urological Research Group on hormone therapy for prostatic tumours is based on the high incidence of cardiovasuclar side effects seen after long-term oestrogen therapy in these patients and underlines the need for other forms of medical treatment in the advanced disease.

Since prolactin is known to have a stimulating effect on prostatic growth and function (7, 10), anti-prolactins such as ergot alkaloids have recently been introduced into palliative treatment protocols for advanced cancer of the prostate (4, 6).

Bromocriptine (CB154) significantly decreases the uptake of dihydrotestosterone by the hypertrophic prostate, and testosterone by carcinomatous prostate (1, 12). This effect is reversed by previous castration in the dog (11). The intraprostatic metabolic changes are dose dependent and not related to prolactin suppression. Whereas 7.5 mg bromocriptine per day results in a marked increase of androgen uptake, the high dosage of 15 mg daily causes a significant depression of androgen uptake by prostate tissue (1).

In a clinical trial, Coune and Smith administered bromocriptine in the low dose of 7.5 mg per day to 25 patients with advanced carcinoma of the prostate over 8 weeks (4). These patients were in part already treated by hormone manipulation and resistant to testosterone depletion. In no case could improvement of all objective parameters be observed. These controversial results were probably predictable in the light of our unpublished findings that low-dose bromocriptine increases androgen uptake by

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prostate tissue, whereas with higher doses, beneficial effects have been observed.

In the present study an attempt has been made to quantify the metabolic changes induced after prolactin suppression by bromocriptine. Since the dose of 15 mg bromocriptine per day was found to inhibit testosterone uptake in patients with carcinoma of the prostate, the same dose was administered for the study of plasma kinetics.

As a control, ethinyl oestradiol was also investigated. A low dose of ethinyl oestradiol (0.6 mg/day) was chosen because (1) the dose of oestrogen should ensure minimal side effects and (2) the dose should still be high enough to suppress testosterone secretion as much as bromocriptine. It must be pointed out, however, that the ideal dose of exogenous oestrogen remains undetermined (5), and the effective dose seems to be dependent on the tumour stage (15).

MATERIAL AND METHODS

Subjects

Twenty-five patients, all informed volunteers, were studied. All tumours were stage C and D lesions without previous antitumour treatment. Histological confirmation and grade of differentiation of the carcinoma were obtained by trans-rectal needle biopsy prior to the study and after a 5-day regimen of the drug under investigation. Immediately prior to and during the investigation, no other medication was taken which might influence steroid metabolism. None of the patients selected had a history of cardiovascular, thromboembolic or hepatic disease.

Materials

(1, 2 ^{3}H)-Testosterone (S. A. 58 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks, England and was at least 95 % pure when examined by thin layer chromatography in the system hexane: methanol : H_2O (5 : 4 : 1; v/v/v). The non-radioactive reference steroids and TLC Silica Gel plastic sheets F 254 were from E. Merck, Darmstadt, West Germany. All organic solvents were of analytical grade. Bromocriptine (CB 154) was kindly supplied by Sandoz AG; Basle, Switzerland and ethinyl oestradiol (Progynon M) was purchased from Schering AG, Berlin, West Germany.

Test Procedure

After an overnight fast the study was started at 8.00 am. Initial blood samples were taken for determination of plasma testosterone, serum prolactin, LH and FSH by means of radioimmunoassay. ^{3}H-testosterone (58 Ci/mmol) was injected intravenously and blood samples were drawn at intervals up to 5 hours. In the oestrogen group, 5 patients received ethinyl oestradiol 0.6 mg daily over 5 days and an equal number served as controls. In the anti-prolactin group, 15 patients were given bromocriptine 15 mg daily and underwent post-treatment study. Determination of radioactive androgen metabolites by thin layer chromatography was performed as described previously (18).

Plasma Kinetics

The single injection technique was used for the study of plasma kinetics, and kinetic parameters were calculated from a computer programme based on the two-compartment model described by Tait et al. (23). This model represents the two main distribution volumes and interchanges of a hormone secreted physiologically and injected. The mathematically defined kinetic parameters are calculated according to a Taylor regression. The inner pool volume "V_1" represents androgen bound to plasma proteins and metabolised within the liver. "V_2", the outer pool volume, is characterised by androgen accumulated by hormone sensitive tissues and can be defined as the entity of enzymes capable of metabolising androgens not confined to organ boundaries. This distribution volume has been characterised by Braunsberg as the "deep compartment" (3). "K_1" represents a rate constant, the transfer from the inner to the outer pool, and "K_2" the rate of metabolism within the inner pool. When the radioactivities in plasma are plotted on a semilogarithmic scale against time, the plasma disappearance curve of the hormone is obtained: α is the slope of the initial fast component, β the slope of the final slow component of the curve. Thus, the elimination of hormone from the application compartment can be defined and the metabolic clearance rate can be calculated by determining the area under the disappearance curve. Finally, the biological half-life (T1/2) of the compound under study is calculated. Values are expressed as mean $\pm$ standard error of the mean (SEM) and significance is determined by Student's T test.

RESULTS

Endogenous Hormones

Bromocriptine: Prolactin decreased from 12.4 ± 1.2 ng/ml (SEM) to 3.7 ± 0.8 ng/ml ($p < 0.001$) after a 5-day treatment (Fig. 1). Plasma testosterone decreased from 422.5 ± 30.7 ng % to 265.3 ± 26.5 ng % ($p < 0.01$). A moderate elevation of serum LH was found, whereas bromocriptine had no effect on pituitary FSH release.

Ethinyl Oestradiol: LH decreased from 25.9 ± 3.8 mU/ml to 14.6 ± 0.8 mU/ml ($p < 0.1$), FSH from 22.6 ± 8.9 mU/ml to 10.1 ± 2.0 mU/ml ($p < 0.2$), and plasma testosterone from 467 ± 106 ng % to 184 ± 67 ng % ($p < 0.01$).

Plasma Kinetics

The initial fast component of testosterone disappearance (slope α) is not altered by either oestrogen or anti-prolactin (Fig. 2). Bromocriptine, however, markedly enhanced the elimination of testosterone. The slope β of the final part of the curve increased from 9.7 ± 1.7 U/d to 16.1 ± 4.5 U/d after bromocriptine as compared to the controls ($p < 0.2$). After ethinyl oestradiol testosterone elimination was prolonged compared to the controls, with a significant decline of the slope β of the final component of the curve ($p < 0.002$). In accordance with the altered testosterone elimination and decreased (bromocriptine) or prolonged (oestrogen) duration of androgen within the various compartments, the overall interconversion of androgens is influenced by the rate of appearance of testosterone metabolites with time (Fig. 3).

The prolonged elimination time for androgen after oestrogen is also reflected by the slightly increased outer pool volume V_2 (Fig. 4). The transfer of hormone from the inner pool into the deep compartment (K_1) is decreased ($p < 0.02$; Fig. 5). Metabolism within the inner pool (K_2) remains unchanged.

The faster elimination of testosterone following bromocriptine administration matches the increase of the inner pool metabolism (K_2) and the diminished transfer of testosterone into the deep compartment (Fig. 6A). The outer pool itself is strikingly reduced (Fig. 4). Consequently, the metabolic clearance rate, i.e. volume of plasma totally and irreversibly cleared per unit time, is increased whereas the biological half-life is shortened. Oestrogen treatment causes a significantly prolonged biological half-life as a result of the longer duration of the injected testosterone ($p < 0.025$; Fig. 6B).

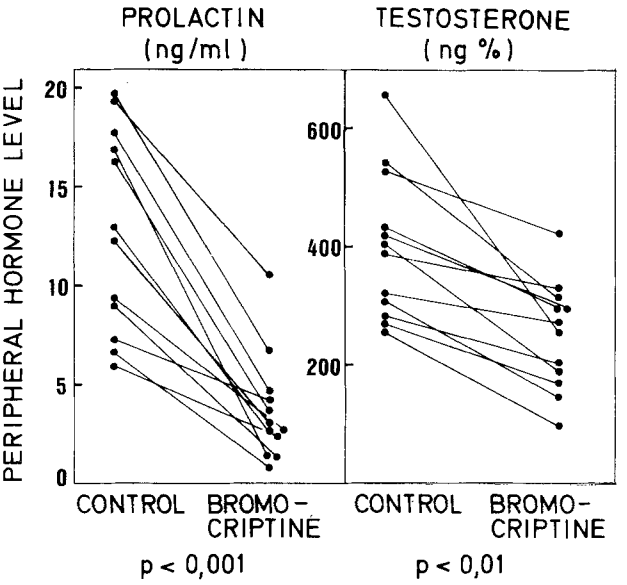


Fig. 1. Effect of bromocriptine on serum prolactin and plasma testosterone (n = 12). Hormones were measured by radioimmunoassay before and after a 5-day regimen of 15 mg per day

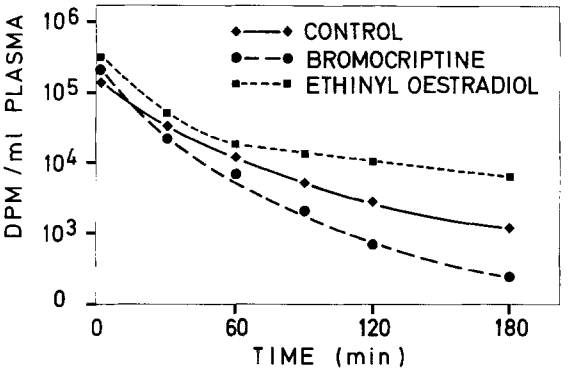


Fig. 2. The effect of bromocriptine and ethinyl oestradiol on the elimination of labelled androgen from plasma. After injection of ^{3}H-testosterone blood samples were drawn at various time intervals, androgen metabolites separated by TLC and assayed for radioactivity in a liquid-scintillation counter

DISCUSSION

The investigations described have been designed to clarify the mode of action by which the anti-prolactin bromocriptine may have beneficial effects in patients with prostatic carcinoma. Therefore, its influence

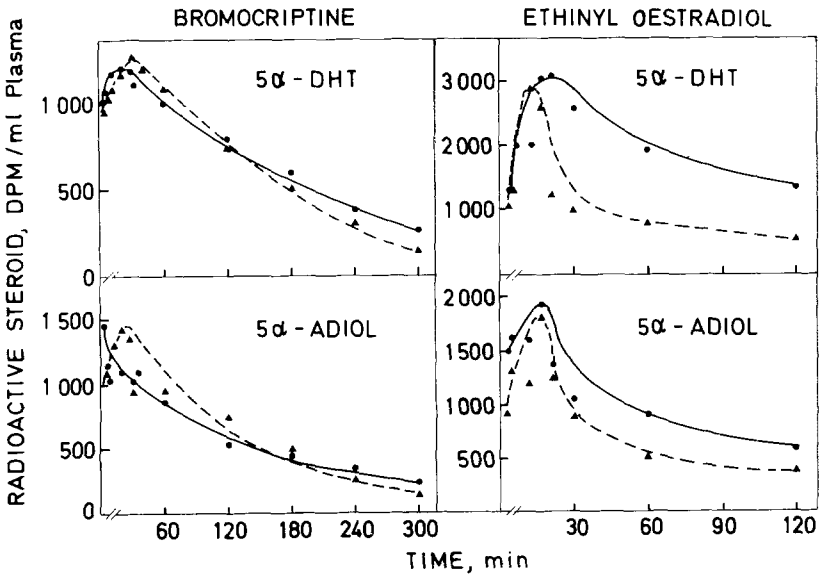


Fig. 3. The effect of bromocriptine and ethinyl oestradiol on the appearance of labelled testosterone metabolites with time; DHT = Dihydrotestosterone, Adiol = Androstane-3 α-17 β-diol, ●—● before treatment, ▲---▲ after treatment

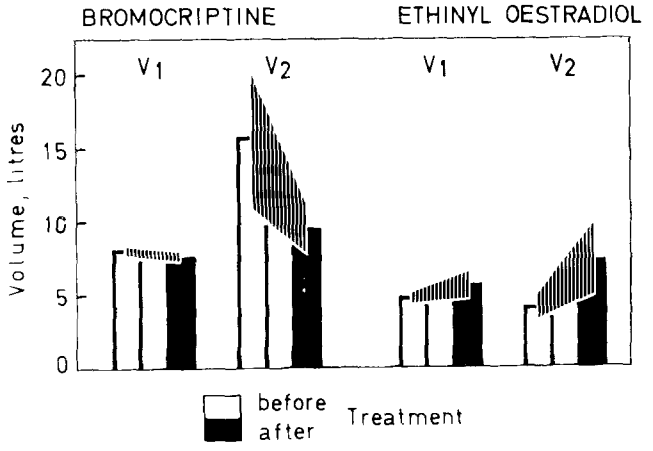


Fig. 4. The effect of bromocriptine and ethinyl oestradiol on the inner pool volume (V_1) and outer pool volume (V_2) of androgen; shaded area = SEM

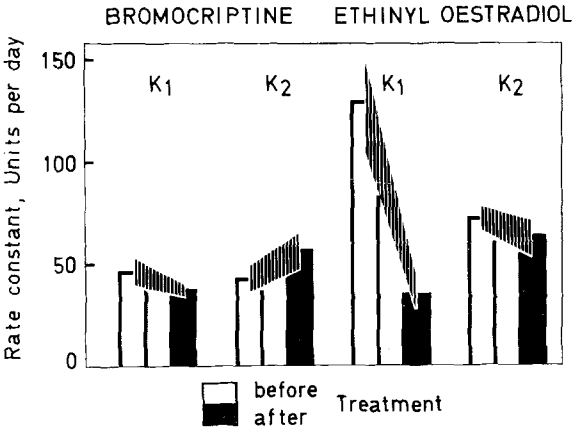


Fig. 5. The effect of bromocriptine and ethinyl oestradiol on the rate constants of androgen kinetic metabolism; K_1 = transfer rate from inner to outer pool, K_2 = rate of metabolism within the inner pool, shaded area = SEM

on testosterone secretion was studied in untreated individuals. Bromocriptine did not only suppress plasma testosterone, but also resulted in a markedly faster elimination of androgens.

The second aim was to compare the kinetic effects of bromocriptine with an established oestrogen, ethinyl oestradiol. To eliminate the fact that a high dose of oestrogen might interfere with the experimental model by causing a higher degree of testosterone suppression, the low dose of 0.6 mg ethinyl oestradiol which is a known to produce a similar degree of testosterone depression (22) was chosen. 15 mg bromocriptine has an equivalent effect on testosterone metabolism though it must be emphasised that these doses are not equimolar.

In interpreting the effect of bromocriptine on endogenous hormone levels and on the kinetic behaviour of testosterone, it is evident that (a) prolactin and testosterone, both stimulators of prostatic growth, are depressed; (b) bromocriptine inhibits the transfer of androgen from the inner pool into the deep compartment, and (c) it favours the degradation of androgen before entering target tissues.

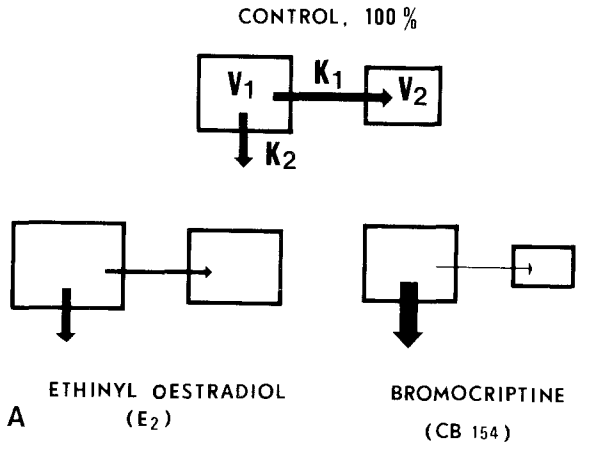
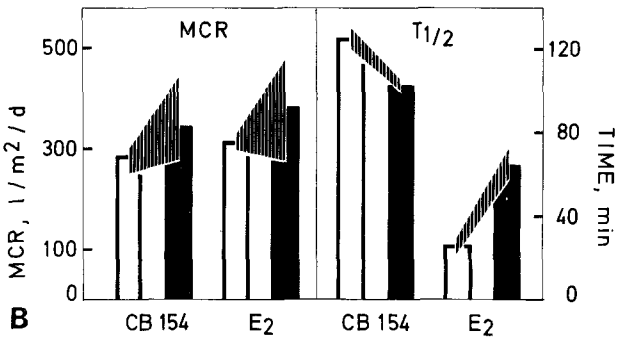


Fig. 6. A) Changes of kinetic parameters of ^{3}H-testosterone after bromocriptine and ethinyl oestradiol according to the two-compartment model as compared to pre-treatment values (100 %). Kinetic parameters are calculated from a computer programme: abbreviations as indicated in Figures 4 and 5.



B) Metabolic clearance rate (MCR) and biological half-life (T 1/2) of testosterone after bromocriptine and ethinyl oestradiol as computed from the disappearance of androgen by a Taylor regression

The net effect of the more pronounced elimination of hormone along with the documented kinetic changes is a diminished amount of androgen available for entry into the prostate. Our unpublished observations that androgen uptake by carcinomatous tissue is diminished after bromocriptine would further support our hypothesis in that the lowered prolactin level causes intra- and extra-prostatic changes of the androgenic hormone milieu essential for prostate growth and function.

No definite conclusions can be drawn, however, as to whether prolactin suppression per se or bromocriptine per se is responsible for the apparent prostatic metabolic alterations. Since the hepatic synthesis of prolactin receptors is influenced by androgens (2, 13) and by the peripheral prolactin level (20, 21), the prostatic effect of bromocriptine could in part be due to a diminished amount of receptor protein attached to the cell surface (14). Furthermore, it remains unclear to what extent the plasma binding proteins for testosterone are altered by prolactin suppression, thus influencing the rate of distribution of the injected radioactive androgen into the compartments.

From this study it is evident that the known local and systemic beneficial effect of oestrogen on prostatic malignancies cannot be accomplished by plasma kinetic changes of endogenous androgens. Testosterone, although suppressed to the same degree as bromocriptine, persisted for longer after oestrogen. As shown by other investigators, oestrogens increase prolactin secretion by a feedback to hypothalamic and pituitary centres (9). Although not measured in this study, elevated levels of peripheral prolactin after ethinyl oestradiol might well account for the adverse effect in the kinetic behaviour of testosterone after oestrogen when compared to bromocriptine administration.

It could even be argued that the initial unresponsiveness to testosterone depletion, or the acquired failure to respond to oestrogen treatment seen especially in patients with poorly differentiated lesions, is at least in part due to prolactin elevation with concomitant activation of prostatic androgen metabolism. It is also conceivable that the major extraprostatic effect of oestrogen along with the known decline of the testosterone production rate (17) is reflected by the induction of peripheral sex hormone binding globulin (19, 24). Thus the testosterone "free index" would be lowered and the amount of androgen capable of entering the prostate diminished.

In conclusion, bromocriptine suppresses prolactin and the endogenous testosterone secretion in patients with untreated advanced cancer of the prostate. Furthermore, it favours the elimination of injected labelled testosterone by decreasing the transfer of hormone into the deep compartment and by stimulating the inner pool androgen degradation. A low but (with respect to testosterone suppression) equivalent dose of ethinyl oestradiol caused persistance of the injected

androgen with a prolonged biological half-life for testosterone. While the beneficial effects of prolactin suppressing compounds observed in patients with prostatic cancer can be explained by the reduced amount of androgen available to the prostate, the extraprostatic effects of oestrogen cannot be explained by the alterations in the plasma kinetic metabolism of testosterone.

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REFERENCES

1. Altwein, J.E., Sinterhauf, K., Hutschenreiter, G.: Antiprolactine und Prostataadenom: Untersuchung eines neuen therapeutischen Prinzips. In: Verhandlungsbericht der Deutschen Gesellschaft für Urologie. Berlin-Heidelberg-New York: Springer 1977
2. Aragona, C., Friesen, H.G.: Specific prolactin binding sites in the prostate and testis of rats. Endocrinology 97, 677 (1975)
3. Braunsberg, H.: Mathematical models in the study of steroid dynamics. Advances in Biological Sciences 5, 145 (1969)
4. Coune, A., Smith, P.: Clinical trial of 2-bromo-α-ergocryptine (NSC-169774) in human prostatic cancer. Cancer Chemotherapy Report 59, 209 (1975)
5. De Vere White, R., Paulson, D.F., Glenn, J.F.: The clinical spectrum of prostate cancer. Journal of Urology 117, 323 (1977)
6. Farnsworth, W.E.: Prolactin and the prostate. In: Prolactin and Carcinogenesis. 4th Tenovus Workshop. Eds. Boyns, A.R., Griffiths, K., Cardiff, Alpha Omega Alpha Publishing 1972
7. Farnsworth, W.E.: Role of lactogen in prostatic physiology. Urological Research 3, 129 (1975)
8. Flocks, R.H.: The treatment of stage C prostatic cancer with special reference to combined surgical and radiation therapy. Journal of Urology 109, 461 (1973)
9. Frantz, A.G., Kleinberg, D.L., Noel, G.L.: Studies on prolactin in man. Recent Progress in Hormone Research 28, 527 (1972)
10. Grayhack, J.T.: Pituitary factors influencing growth of the prostate. National Cancer Institute Monographs 12, 189 (1963)
11. Helmerich, D., Altwein, J.E.: Effect of prolactin and the anti-prolactin bromocripitine on the testosterone uptake and metabolism in androgen-sensitive and insensitive canine organs. Urological Research 4, 101 (1976)
12. Jacobi, G.H., Sinterhauf, K., Kurth, K.H., Altwein, J.E.: Bromocriptine and prostatic carcinoma: Plasma kinetics, production and tissue uptake of ^{3}H-testosterone in vivo. Journal of Urology (in press)
13. Kelly, P.A., Posner, B.I., Friesen, H.G.: Effects of hypophysectomy, ovariectomy and cycloheximide on binding sites for lactogenic hormones. Clinical Research 22, 732 (1974)
14. Kledzik, G.S., Marshall, S., Campbell, G.A., Gelato, M.: Effects of castration, testosterone, estradiol and prolactin on specific prolactin-binding activity in ventral prostate of male rats. Endocrinology 98, 373 (1976)
15. Kent, J.R., Bischoff, A.J., Arduino, L.J., Mellinger, G.T., Byar, D.P., Hill, M., Kozbur, X.: Estrogen dosage and suppression of testosterone levels in patients with prostatic carcinoma. Journal of Urology 109, 858 (1973)
16. Ludvick, W., Riedl, P., Zaunbauer, W.: Beeinflußbarkeit des Prostatakarzinoms durch Östrogene. Zeitschrift für Urologie 66, 165 (1974)
17. Nolten, W.E., Sholiton, L.J., Srivastava, L.S., Knowles, Jr., H.C., Werk, Jr., E.E.: The effects of diethylstilbestrol and medroxyprogesterone acetate on kinetics and production of testosterone and dihydrotestosterone in patients with prostatic carcinoma. Journal of Clinical Endocrinology and Metabolism 43, 1226 (1976)
18. Orestano, F., Altwein, J.E., Knapstein, P., Bandhauer, K.: Mode of action of progesterone, gestonororone capronate (Depostat) and cyproterone acetate (Androcur) on the metabolism of testosterone in human prostatic adenoma: in vitro and in vivo investigations. Journal of Steroid Biochemistry 6, 845 (1975)
19. Pearlman, W.H., Crépy, O., Murphy, M.: Testosterone binding levels in serum of women during the normal menstrual cycle, pregnancy and post-partum period. Journal of Clinical Endocrinology and Metabolism 27, 1012 (1967)
20. Posner, B.I., Kelly, P.A., Friesen, H.G.: Induction by a polypeptide hormone of its own receptor. Clinical Research 22, 733 (1974)
21. Posner, B.I., Kelly, P.A., Friesen, H.G.: Induction of a lactogenic receptor in rat liver: Influence of estrogen and the pituitary. Proceedings of the National Academy of Sciences 71, 2407 (1974)
22. Shearer, R.J., Hendry, W.F., Sommerville, I.F., Fergusson, J.D.: Plasma testoster-

one: An accurate monitor of hormone treatment in prostatic cancer. British Journal of Urology 45, 668 (1973)
23. Tait, J. F., Tait, S. A. S., Little, B., Laumas, K. R.: The disappearance of 7-H^3-d-aldosterone in the plasma of normal subjects. Journal of Clinical Investigation 40, 72 (1961)
24. Tochimoto, S., Olivo, J., Southern, A. L., Gordon, G. G.: Studies of the plasma β-globulin: Sex difference and effect of ethinyl estradiol and testosterone. Proceedings of the Society for Experimental Biology and Medicine 134, 700 (1970)
25. Veterans Administration Cooperative Urological Research Group: Carcinoma of the prostate: treatment comparisons, Journal of Urology 98, 516 (1967)
26. Veterans Administration Cooperative Urological Research Group: Treatment and survival of patients with cancer of the prostate. Surgery, Gynecology and Obstetrics 124, 1011 (1967)
27. Veterans Administration Cooperative Urological Research Group: Factors in the prognosis of carcinoma of the prostate: A cooperative study. Journal of Urology 100, 59 (1968)
28. Whitmore, Jr., W. F., Hilaris, B., Grabstald, H. P., Batata, M.: Implantation of ^{125}I in prostatic cancer. Surgical Clinics of North America 54, 887 (1974)

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