*Journal of Steroid Biochemistry*, 1974, Vol. 5, pp. 609–617. Pergamon Press. Printed in Great Britain

# STUDIES ON THE *IN VIVO* HORMONAL CONTROL OF RAT PROSTATIC TESTOSTERONE 4-ENE-5α-REDUCTASE ACTIVITY*†

DAVID K. H. LEE, CHARLES E. BIRD and ALBERT F. CLARK

Departments of Biochemistry and Medicine, Queen's University and Kingston General Hospital, Kingston, Ontario, Canada

(*Received* 11 *March* 1974)

SUMMARY

Because recent reports indicate that the enzymatic 4-ene-5α-reduction of testosterone is an important step in the expression of androgenic activity in the prostate, we have investigated the effects of several hormonal situations on the activity of this enzyme in adult rat prostate. Both specific (pmol testosterone 5α-metabolites formed/mg protein/60 min) and total (pmol testosterone 5α-metabolites formed/rat prostate/60 min) activities were measured. Experimental groups subjected to castration or adrenalectomy showed decreased specific and total activities when compared to intact controls. In the hypophysectomized control group the specific activity was increased while the total activity was decreased as compared to intact controls. Estradiol administration for four days to each of the four groups resulted in decreases in both specific and total activities when compared to their group controls. Testosterone administration for four days to each of the four groups resulted in increases in both specific and total activities as compared to their group controls. Combined estradiol and testosterone administration, when compared to the effects of testosterone alone, resulted in no essential change in the intact group, an increase in the castrated group and decreases in the hypophysectomized and adrenalectomized groups. Ovine prolactin administration alone or in combination with estradiol or testosterone or both resulted in no consistent change in enzyme activity in either the intact or hypophysectomized groups. Our results suggest that a number of hormones can modify the activity of prostatic testosterone 4-ene-5α-reductase activity and hence may influence the growth and activity of the prostate.

## INTRODUCTION

The elucidation of the primary molecular processes by which testosterone controls the differentiation, growth and maintenance of specific cellular functions in androgen target organs such as the prostate is currently under extensive research. Recent evidence has indicated that although testosterone may be quantitatively the most important naturally occurring androgen, one of its 5α-reduced metabolites, in particular, 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) may be the biologically important intracellular androgen. This metabolite is known to possess potent biological activity [1]. 5α-Dihydrotestosterone has been shown by *in vitro* [2–4] and *in vivo* [4–6] methods to be the principle metabolite of testosterone found in rat prostate. In human hypertrophic prostate, 5α-dihydrotestosterone is formed from testosterone and accumulates at a higher concentration than occurs in the normal gland [7, 8]. Intracellularly, 5α-dihydrotestosterone is found in high concentrations in the rat prostate cell nucleus, and is bound to the chromatin [4]. Such findings led to the belief that the 4-ene-5α-reduction of testosterone to dihydrotestosterone is the primary step in the expression of androgenic activity in target organs.

Estrogen treatment is often used as a form of therapy for prostatic cancer, which can be androgen dependent. However, its complete mode of action is not fully understood. Estrogen may influence the prostate indirectly by decreasing testicular testosterone production; this is achieved by inhibiting gonadotrophin production [9]. Estrogen administration to humans lowers the metabolic clearance rate of testosterone in both normal subjects [10] and in patients with prostatic carcinoma [11]. The clearance of 5α-dihydrotestosterone is similarly affected by estrogen treatment [12]. Direct inhibitory effects on testosterone metabolism by

* This work forms part of a thesis submitted by D.K.H.L. to the Department of Biochemistry, Queen's University for the Ph.D. degree.

† The following abbreviations and trivial names have been used: Tris, Tris (hydroxymethyl) aminonethane; 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; androstanediol, 5α-androstane-3α,17β-diol; 17β-estradiol valerate, 3-hydroxyestra-1,3,5[10]-triene-17β-yl valerate.

the prostate have been demonstrated by Farnsworth[13], Groom *et al.*[14], Leav *et al.*[15], Giorgi *et al.*[16] and Lee *et al.*[17].

This paper reports the results of studies on the effects of estrogen administration on the rat prostate gland *in vivo* in particular, the 4-ene-5α-reduction of testosterone to 5α-dihydrotestosterone. Since the growth and function of the prostate gland is influenced by androgen, adrenocortical hormones [18, 19] and pituitary hormones such as prolactin [20] we also studied the effects of testosterone and prolactin *in vivo* and utilized animals which had been castrated or adrenalectomized or hypophysectomized.

## MATERIALS AND METHODS

### *Animals*

Adult male Sprague Dawley rats (minimum body weight 175 g) were obtained from Bio-Breeding of Canada (Ottawa, Canada) and Canadian Breeding Farms and Laboratories Ltd. (CBFL) (Montreal, Canada). Castrated rats were obtained from Bio-Breeding of Canada, adrenalectomized rats from CBFL and hypophysectomized rats from Carworth Laboratories Ltd. (New City, New York, U.S.A.) and CBFL. Castration and hypophysectomy were performed twelve days and adrenalectomy seven days before the start of the experiments. All rats were kept in a temperature and humidity controlled environment. Standard rat food was given to intact and castrated rats while 5% glucose in 0·9% NaCl was given to adrenalectomized and hypophysectomized rats and their corresponding intact control animals. All food and drink were given *ad libitum*.

### *Chemicals*

[1,2-$^3$H]-Testosterone (50 Ci/mmol), [4-$^{14}$C]-testosterone (50 mCi/mmol) and [4-$^{14}$C]-dihydrotestosterone (56·1 mCi/mmol) were obtained from New England Nuclear Corporation (Boston, Mass.). [4-$^{14}$C]-androstanediol was prepared from [4-$^{14}$C]-dihydrotestosterone by reduction in the presence of excess NADH with 3α-hydroxysteroid:NAD oxidoreductase purified from a dried cell preparation of steroid induced pseudomonas testosteroni (Winley–Morris Co., Montreal, Canada), as described by Delin *et al.*[21]. All of these steroids were at least 97% pure as determined by paper chromatography and the reverse isotope dilution procedure.

Non-radioactive steroids were obtained as follows: testosterone, 5α-dihydrotestosterone, androstanediol (5α-androstane-3α,17β-diol) and estradiol, from Sigma Chemical Company (St. Louis, Mo., U.S.A.); estradiol valerate (Delestrogen), testosterone enanthate (Delatestryl) and estradiol valerate–testosterone enanthate (Deladumone 2×) from E. R. Squibb and Sons Ltd. (Montreal, Canada).

Ovine prolactin, bovine serum albumin, calf thymus DNA and NADPH were obtained from Sigma Chemical Company. All other chemicals were of analytical or reagent grade. Solvents were distilled before use.

### *Administration of estrogens* in vivo

Estradiol was given either as estradiol itself or as estradiol valerate (Delestrogen). The dosages used were 0·1 mg estradiol in 0·2 ml propylene glycol/rat/day or 0·1 mg estradiol valerate (Delestrogen) in 0·1 ml sesame oil/rat/day. The dosage for testosterone administration was 2·25 mg of testosterone enanthate (Delatestryl) in 0·1 ml sesame oil/rat/day. In cases where both estradiol valerate and testosterone enanthate were given, they were administered as a mixture (Deladumone 2×) in 0·1 ml sesame oil, the amount of each being the same as when given individually. Ovine prolactin was dissolved in 0·9% NaCl buffered to pH 9·0 with glycine buffer. The prolactin dosage was 300 μg in 0·2 ml buffered 0·9% NaCl/rat/day. Control animals were given the same quantity of the corresponding vehicle. All injections were given intraperitoneally.

### *Tissue preparation*

The animals were killed by decapitation and the prostate glands were immediately removed and immersed in ice cold 0·01 M Tris–HCl buffer (pH 7·4) containing EDTA ($5·0 \times 10^{-5}$ M), $MgCl_2$ ($5·0 \times 10^{-3}$), mercaptoethanol ($5·0 \times 10^{-4}$ M) and NaCl ($1·5 \times 10^{-2}$ M). Homogenates, nuclear and microsomal fractions were prepared as described by Lee *et al.*[17]. The nuclear fraction was purified by the method of Maggio *et al.*[22] as modified by Bruchovsky and Wilson[4]. For all experimental groups of animals, the prostate glands were pooled, weighed together and this total weight was divided by the number of animals to give the "mean prostate weight" results listed in the Tables.

### *Assays*

The prostatic 4-ene-5α-reductase activity was measured as described by Lee *et al.* [17]. Essentially, [1,2-$^3$H]-testosterone ($2·8 \times 10^{-9}$ M) was incubated with tissue preparations and the metabolites, $^3$H-5α-dihydrotestosterone and $^3$H-androstanediol, were isolated by thin layer and paper chromatography. Losses of compounds during isolation procedures were monitored by $^{14}$C-labelled steroids and the final $^3$H counts were corrected accordingly. The combined $^3$H counts in 5α-dihydrotestosterone and androstanediol were used as a measure of 4-ene-5α-reductase activity, which is

Table 1. Effects of estradiol administration (0·1 mg/day intraperitoneally for 0–16 days) on body weight, prostate weight and prostate DNA and protein contents in intact adult rats

| Days of administration | Mean body weight (g) | Mean prostate weight (mg) | Prostate DNA/ rat (mg) | Prostate protein per rat (mg) | | |
|---|---|---|---|---|---|---|
| | | | | Total | Nuclear* | Microsomal |
| 0(10)† | 210 | 200 | 0·6 | 17·0 | 9·3 | 0·5 |
| 2(5) | 216 | 200 | 0·8 | 20·9 | — | — |
| 4(23) | 203 | 138 | 0·7 | 10·7 | 3·4 | 0·4 |
| 6(4) | 222 | 111 | — | 14·0 | — | — |
| 8(5) | 189 | 108 | 0·5 | 10·0 | — | — |
| 16(8) | 195 | 112 | 0·4 | 7·7 | 1·1 | 0·3 |

* Nuclear protein content corrected for losses from DNA results.
† Number of animals in group.

expressed either as specific activity (pmoles of 5α-metabolites formed/mg protein/60 min) or as total activity per prostate (pmol of 5α-metabolites formed/prostate/60 min).

Total protein was determined by the method of Miller[23] which is a modification of the method of Lowry *et al.*[24]. Bovine serum albumin was used as a standard. All protein determinations were done in duplicate; all prostatic protein results in the Tables are the averages of these duplicate determinations. The range of results above and below the average were never greater than 15%. DNA was extracted by the method of Munro and Fleck[25] and measured colorimetrically by the method of Burton[26]. Calf thymus DNA was used as standard. Only single determinations of DNA were performed.

## RESULTS

### *Studies on intact rats*

*Effects of estradiol.* In these studies, each rat was given daily intraperitoneal injections of 0·1 mg of estradiol. Animals were killed at selected times up to 16 days and the prostates were removed for analysis.

As shown in Table 1 and Fig. 1, estradiol administration for 2 days did not produce any significant changes in the parameters studied. After 4 days, there were decreases in the prostate weight and its protein content. A decrease in prostate DNA content was observed only after 8 days. The specific activity of 4-ene-5α-reductase in the homogenate was not changed after 2 days, while it was decreased at 4 days and at 6 days had reached a plateau. The total enzyme activity continued to decline throughout the 16 days.

The nuclear and microsomal fractions were studied only at 4 and 16 days of estradiol administration. The results are shown in Fig. 2. Both fractions showed a steady decrease for total enzyme activity as did the homogenate. The greater decrease in nuclear protein content than in microsomal protein (Table 1) accounts for an increase in nuclear enzyme specific activity after 16 days of estrogen administration.

### *Effects of estradiol valerate, testosterone enanthate and ovine prolactin*

Animals in these studies were given either individual hormones or combinations of hormones. Hormones were administered for 4 days and the results are shown in Table 2 and Fig. 3.

As seen in Table 2, the body weights were not greatly different for the different groups. The prostate weight decreased following estradiol administration either with or without simultaneous prolactin administration. It increased after testosterone administration; the increases were smaller when either

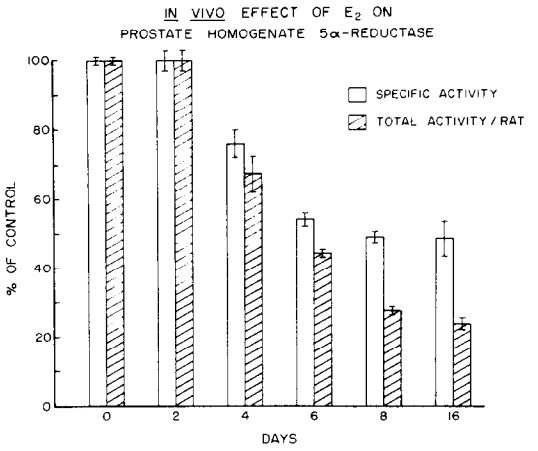


Fig. 1. Effects of *in vivo* administration of estradiol (0·1 mg/day intraperitoneally for 0–16 days) on prostate homogenate testosterone 4-ene-5α-reductase activity in adult Sprague–Dawley rats. Results are expressed as per cent of the 0 day group results (specific activity = 4·60 pmol of 5α-metabolites formed/mg protein/60 min; total activity = 78·3 pmol of 5α-metabolites formed/prostate/60 min). Each result is the average of two assays and the bar lines indicate the range of results.

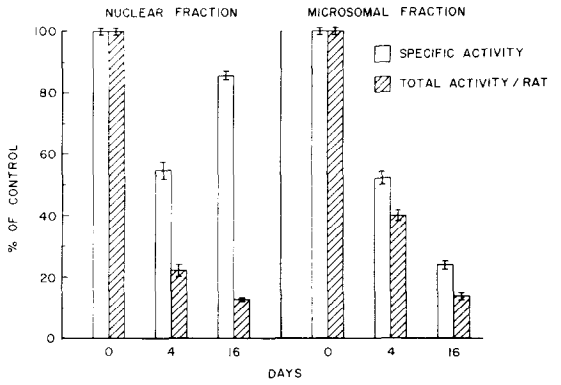


Fig. 2. Effects of *in vivo* administration of estradiol (0·1 mg/day intraperitoneally for 0, 4 and 16 days) on prostate nuclear and microsomal testosterone 4-ene-5α-reductase activity in adult Sprague–Dawley rats. Results are expressed as per cent of the 0 day group results (nuclear fraction: specific activity = 9·10 pmol 5α-metabolites formed/mg protein/60 min; total activity = 85·0 pmol 5α-metabolites formed/prostate/60 min. Microsomal fraction: specific activity = 30·0 pmol 5α-metabolites formed/mg protein/60 min; total activity = 10·0 pmol 5α-metabolites formed/prostate/60 min). Each result is the average of 3 assays and the bar lines indicate the range of results.

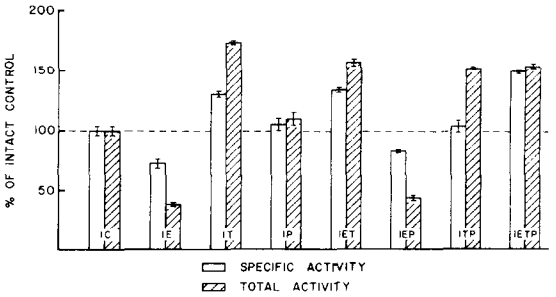


Fig. 3. Effects of *in vivo* administration of estradiol valerate (0·1 mg/day for 4 days), testosterone enanthate (2·25 mg/day for 4 days), ovine prolactin (300 μg/day for 4 days), singly and in combinations on prostate homogenate testosterone 4-ene-5α-reductase activity in intact adult Sprague–Dawley rats. Results are expressed as per cent of intact control results (specific activity = 2·14 pmol of 5α-metabolites formed/mg protein/60 min; total activity = 83·1 pmol of 5α-metabolites formed/prostate/60 min). Each result is the average of 3 assays and the bar lines represent the range of results. IC = intact controls; IE = animals receiving estrogen; IT = animals receiving testosterone; IP = animals receiving prolactin; IET = animals receiving estrogen and testosterone; IEP = animals receiving estrogen and prolactin; ITP = animals receiving testosterone and prolactin; IETP = animals receiving all three hormones.

estrogen or prolactin or both were administered simultaneously with the testosterone. Prolactin by itself had no significant effect on prostatic weight. The prostate DNA content did not show large changes when the hormones were given but increased slightly following the administration of testosterone or prolactin and when all the three hormones were given together. The prostate protein content was increased following testosterone administration and was further increased when prolactin was given simultaneously. Estradiol administration decreased the protein content when given by itself or in combination with prolactin and apparently prevents the increase caused by testosterone and prolactin when the 3 hormones were given together. Prolactin alone did not cause any significant change.

Figure 3 shows the prostate 4-ene-5α-reductase activities (specific and total) in the homogenates in response to the different hormones. Estradiol administration decreased enzyme activity while testosterone increased it. Prolactin alone has no effect. When estradiol and testosterone were given together, the

Table 2. Effects of estradiol valerate (0·1 mg/day for 4 days), testosterone enanthate (2·25 mg/day for 4 days) and ovine prolactin (300 μg/day for 4 days) on body weight, prostate weight and prostate DNA and protein contents in intact adult rats

| Animal group | No. of animals in each group | Mean body weight (g) | Mean prostate weight (mg) | Prostate DNA/rat (mg) | Prostate protein/rat (mg) |
|---|---|---|---|---|---|
| Intact | | | | | |
| Control | 6 | 260 | 350 | 1·8 | 38·9 |
| Estradiol | 7 | 221 | 230 | 1·7 | 20·4 |
| Testosterone | 6 | 268 | 430 | 2·3 | 51·7 |
| Prolactin | 6 | 260 | 360 | 2·5 | 40·6 |
| Estradiol + Testosterone | 7 | 243 | 390 | 2·2 | 45·5 |
| Estradiol + Prolactin | 6 | 231 | 220 | 1·6 | 16·2 |
| Testosterone + Prolactin | 6 | 243 | 360 | 1·7 | 71·4 |
| Estradiol + Testosterone + Prolactin | 6 | 248 | 390 | 2·5 | 39·9 |

Table 3. Effects of estradiol valerate (0·1 mg/day for 4 days) and testosterone enanthate (2·25 mg/day for 4 days) administration on body weight, prostate weight and prostate DNA and protein contents in adult rats castrated 12 days prior to the start of the experiment

| Animal group | No. of animals in each group | Mean body weight (g) | Mean prostate weight (mg) | Prostate DNA/ rat (mg) | Prostate protein rat (mg) |
|---|---|---|---|---|---|
| Intact Control | 6 | 259 | 333 | 1·4 | 29·3 |
| Castrated Control | 23 | 250 | 35 | 0·1 | 1·2 |
| Castrated + Estradiol | 23 | 231 | 29 | 0·1 | 1·1 |
| Castrated + Testosterone | 18 | 237 | 111 | 0·6 | 9·3 |
| Castrated + Estradiol + Testosterone | 18 | 241 | 106 | 0·5 | 8·2 |

enzyme activity was increased; the same was observed when testosterone was given with prolactin or prolactin plus estradiol. The enzyme activity after prolactin and estradiol administration was not greatly different from that observed after estradiol administration alone.

*Studies on castrated rats*

*Effects of estradiol valerate and testosterone enanthate.* The dosage schedules for the hormones used were the same as for the last experiment. The results are shown in Table 3 and Fig. 4.

While body weights were not significantly affected, removal of the principal source of androgens results in atrophy of the prostate as shown by the prostate weights and prostate DNA and protein contents in Table 3. Estradiol administration did not result in a significant change in the prostate weight and DNA and protein contents as compared to the castrated controls. Testosterone administration increased all the parameters. The combined administration of estradiol and testosterone gave essentially the same result as obtained with testosterone alone.

Figure 4 shows the results for 4-ene-5α-reductase activities. Castration resulted in a great loss of total enzyme activity, but less so in enzyme specific activity. Estradiol administration decreased the activities further. Testosterone administration restored the specific activity to a normal level and total enzyme activity to about one-third of normal. Simultaneous treatment with estradiol and testosterone showed a synergistic effect in that the enzyme specific activity was increased to 140% of the intact control level, while the total enzyme activity was about the same level as obtained with testosterone treatment alone.

*Studies on adrenalectomized rats*

*Effects of estradiol valerate and testosterone enanthate.* The dosage schedules were as described for the last two experiments. The results are shown in Table 4 and Fig. 5.

From Table 4, it can be seen that adrenalectomy resulted in increases in prostate weight and prostate protein content, over those of intact control values. Estradiol administration decreased these parameters

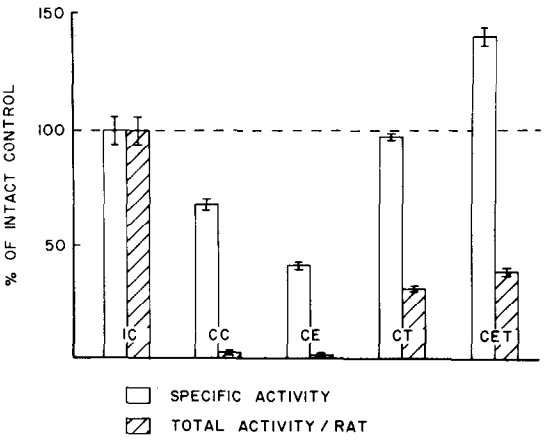


Fig. 4. Effects of *in vivo* administration of estradiol valerate (0·1 mg/day for 4 days) and testosterone enanthate (2·25 mg/day for 4 days) both singly and together on prostate homogenate testosterone 4-ene-5α-reductase activity in adult Sprague–Dawley rats castrated 12 days prior to the start of the experiment. Results are expressed as per cent of intact control animal results (specific activity = 5·86 pmol of 5α-metabolites formed/mg protein/60 min; total activity = 171·8 pmol of 5α-metabolites formed/prostate/60 min). Each result is the average of 2 assays and the bar lines represent the range of results. IC = intact controls; CC = castrated controls; CE = castrated rats receiving estrogen; CT = castrated rats receiving testosterone; CET = castrated rats receiving both.

Table 4. Effects of estradiol valerate (0·1 mg/day for 4 days) and testosterone enanthate (2·25 mg/day for 4 days) administration on body weight, prostate weight and prostate DNA and protein contents in adult rats adrenalectomized 7 days prior to the start of the experiment

| Animal group | No. of animals in each group | Mean body weight (g) | Mean prostate weight (mg) | Prostate DNA/ rat (mg) | Prostate protein/ rat (mg) |
|---|---|---|---|---|---|
| Intact | 3 | 245 | 240 | 1·4 | 22·1 |
| Adrenalectomized Control | 5 | 261 | 470 | 1·5 | 31·8 |
| Adrenalectomized + Estradiol | 8 | 229 | 290 | 1·0 | 17·1 |
| Adrenalectomized + Testosterone | 5 | 235 | 510 | 1·5 | 32·5 |
| Adrenalectomized + Estradiol + Testosterone | 7 | 228 | 490 | 1·2 | 32·4 |

while testosterone administration increased them, although not significantly over the values for the adrenalectomized controls. Estradiol plus testosterone administration did not give results different from those obtained for the adrenalectomized control animals.

Figure 5 shows the effects on 4-ene-5α-reductase activity. Adrenalectomy caused a decrease in enzyme activities, the decrease being greater for specific activity than for total activity. This is probably due to the increase in prostate weight and protein content. Estradiol administration decreased the activities while testosterone administration increased them over the control values. When both estradiol and testosterone were given together, the enzyme activities were not different from those obtained for adrenalectomized control animals.

*Studies on hypophysectomized animals*

*Effects of estradiol valerate, testosterone enanthate and ovine prolactin.* Hypophysectomy greatly decreases the levels of hypophysial, adrenal and gonadal hormones which may affect or mask the estradiol and testosterone effects. The dosage schedules for all three hormones were the same as those described previously for intact rats and the results are shown in Table 5 and Fig. 6.

As expected, there was a great decrease in the size of the prostate following hypophysectomy (Table 5). There were corresponding decreases in prostate protein and DNA contents. Testosterone alone or with estrogen and/or prolactin increased the results for the parameters studied while estrogen alone or prolactin alone or combined had no effect when compared to the hypophysectomized control.

When the prostatic 4-ene-5α-reductase activity was studied (Fig. 6), it was found that hypophysectomy resulted in nearly a complete loss of total enzyme activity; however, the specific activity of the residual enzyme was slightly higher than the intact control value. Estradiol administration decreased the specific activity to the intact control level, with no effect on total activity. Testosterone treatment increased the specific activity to 500% of the intact control values;

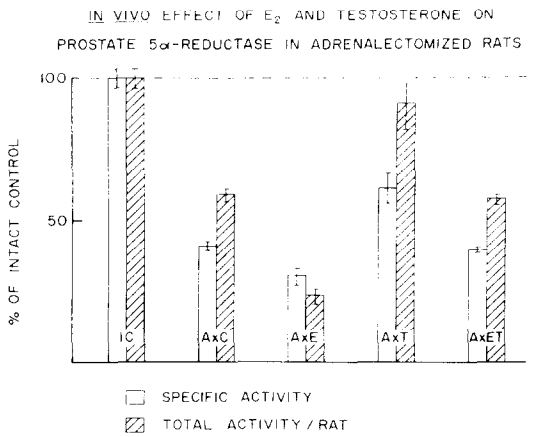


Fig. 5. Effects of *in vivo* administration of estradiol valerate (0·1 mg/day for 4 days) and testosterone enanthate (2·25 mg/day for 4 days) both singly and together on prostate homogenate testosterone 4-ene-5α-reductase activity in adult Sprague–Dawley rats adrenalectomized 7 days prior to the start of the experiment. Results are expressed as per cent of intact control animal results (specific activity = 8·81 pmol of 5α-metabolites formed/mg protein/60 min; total activity = 196·8 pmol of 5α-metabolites formed/prostate/60 min). Each result is the average of 3 assays and the bar lines represent the range of results. IC = intact control; AxC = adrenalectomized control; AxE = adrenalectomized rats receiving estradiol valerate; AxT = adrenalectomized rats receiving testosterone enanthate; AxET = adrenalectomized rats receiving both hormones.

Table 5. Effects of estradiol valerate (0·1 mg/day for 4 days), testosterone enanthate (2·25 mg/day for 4 days) and ovine prolactin (300 μg/day for 4 days) on body weight, prostate weight and prostate DNA and protein contents in adult rats hypophysectomized 12 days prior to the start of the experiment

| Animal group | No. of animals in each group | Mean body weight (g) | Mean prostate weight (mg) | Prostate DNA/ rat (mg) | Prostate protein/ rat (mg) |
|---|---|---|---|---|---|
| Intact Control | 6 | 260 | 350 | 1·8 | 38·9 |
| Hypophysectomized Control | 18 | 157 | 27 | 0·1 | 1·0 |
| Hypophysectomized + Estradiol | 17 | 146 | 22 | 0·1 | 0·7 |
| Hypophysectomized + Testosterone | 20 | 167 | 81 | 0·7 | 5·7 |
| Hypophysectomized + Prolactin | 18 | 169 | 27 | 0·2 | 1·1 |
| Hypophysectomized + Estradiol + Testosterone | 18 | 157 | 63 | 0·5 | 4·0 |
| Hypophysectomized + Estradiol + Prolactin | 20 | 159 | 23 | 0·2 | 1·0 |
| Hypophysectomized + Testosterone + Prolactin | 19 | 171 | 75 | 0·7 | 5·2 |
| Hypophysectomized + Estradiol + Testosterone + Prolactin | 18 | 160 | 72 | 0·6 | 4·6 |

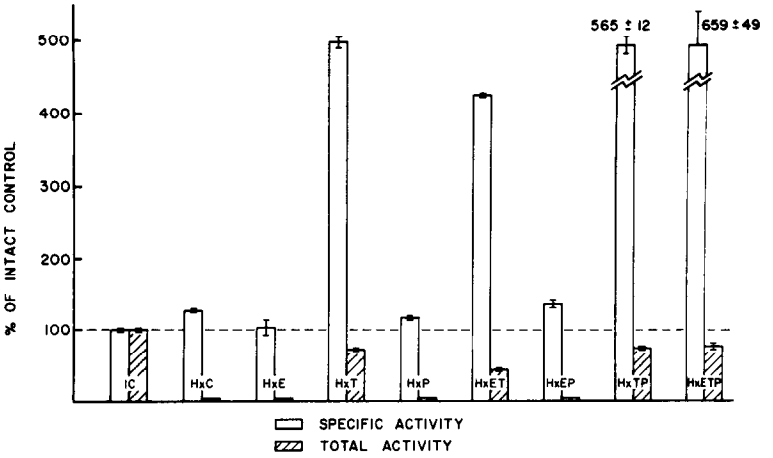


Fig. 6. Effects of *in vivo* administration of estradiol valerate (0·1 mg/day for 4 days), testosterone enanthate (2·25 mg/day for 4 days), ovine prolactin (300 μg/day for 4 days) singly and in combination on prostate homogenate testosterone 4-ene-5α-reductase activity in adult Sprague–Dawley rats hypophysectomized 12 days prior to the start of the experiment. Results are expressed as per cent of intact control results (specific activity = 2·14 pmol of 5α-metabolites formed/mg protein/50 min; total activity = 83·1 pmol of 5α-metabolites formed/prostate/60 min). Each result is the average of 3 assays and the bar lines represent the range of results. IC = intact controls; HxC = hypophysectomized controls; HxE = hypophysectomized rats receiving estrogen; HxT = hypophysectomized rats receiving testosterone; HxP = hypophysectomized rats receiving prolactin; HxET = hypophysectomized rats receiving estrogen plus testosterone; HxEP = hypophysectomized rats receiving prolactin plus estrogen; HxTP = hypophysectomized rats receiving prolactin plus testosterone; HxETP = hypophysectomized rats receiving all three hormones.

but the total activity only to 75%. Prolactin alone did not appear to have any effect. Estradiol and testosterone treatment increased the enzyme activities to a level slightly lower than that with testosterone alone. Prolactin and estradiol treatment resulted in no significant differences from the hypophysectomized control values. When prolactin and testosterone were given together, the enzyme specific activity increased to 565% of intact control, while total activity was increased to 75%, similar to that when testosterone was given alone. When all three hormones were given together, the specific activity was increased even further (659%), while the total activity remained at 75% of the control level.

## DISCUSSION

Although estrogens have been shown to have *in vitro* inhibitory effects on testosterone 4-ene-5α-reduction by the prostate [13–17] their effects *in vivo* are still being investigated. We used estradiol in our experiments since biologically it is the most important naturally occurring estrogen. Commencing after four daily injections of estradiol to normal adult male rats, the prostate protein content and 4-ene-5α-reductase activity decreased (Table 1 and Fig. 1). The DNA content however did not change until the eighth day of estradiol administration. This suggests that the decrease in enzyme activity is a result of an inhibition of protein synthesis. Estradiol *in vitro* has been shown to have no effect on DNA polymerase activity [28]. Since heterogeneous nuclear RNA has a rapid turnover rate, an inhibition of RNA polymerase activity will decrease the amount of protein in the prostate.

If inhibition of protein synthesis is a general phenomenon then a decrease in total 4-ene-5α-reductase activity, but not specific activity, should be observed. Therefore, the decrease in specific activity following estradiol administration suggests the possibility of a selective inhibitory effect on the enzyme. The exact mode of this inhibition remains to be elucidated. The large decrease in nuclear protein content and 4-ene-5α-reductase activity in comparison to the microsomal counterpart (Table 1 and Fig. 2) indicates that the effects may originate in the nucleus. If so, RNA polymerase is certainly a possible target for estradiol action. Our findings of a decrease in the specific activities of 4-ene-5α-reductase activity in the prostate homogenate and nucleii differ from the recently reported data of Moore and Wilson[29] who found no changes in specific activity when 200 μg of estradiol per day was administered for 4 days to intact rats.

Recent findings point to a complex interplay between the pituitary, adrenal and gonadal hormones in the control of growth and function of the prostate gland. There is evidence that prolactin may be involved [20, 30]. Adrenal production of hormones can also affect prostate growth [18, 19]. Our studies with different groups of rats (intact, castrated, adrenalectomized and hypophysectomized) demonstrate that the absence of the pituitary, adrenal, or the gonads can affect prostate growth. Castration alone resulted in a decrease in prostatic protein content, in particular, 4-ene-5α-reductase (Table 3 and Fig. 4). This may be the result of the loss of testosterone produced by the testes. Adrenalectomy increased the prostate protein content and decreased the 4-ene-5α-reductase activity (Table 4 and Fig. 5). Since the prostate glands in this group of rats were presumably still under the influence of testicular testosterone, it appears that the removal of the adrenal hormones changes the response of the prostate to testosterone. Protein synthesis appeared to be stimulated (or protein degradation inhibited) but this did not hold for 4-ene-5α-reductase. Hypophysectomy greatly decreases both the adrenal and gonadal steroid production. Severe atrophy of the prostate was indeed observed (Table 5 and Fig. 6). The slight increase in 4-ene-5α-reductase specific activity was quite different from the response observed after castration and adrenalectomy.

Estradiol treatment to all 4 groups of animals decreased prostatic 4-ene-5α-reductase activity. This may be the result of a decrease in testicular testosterone production. However, following castration and hypophysectomy, there is probably little or no testicular testosterone and therefore the decrease in enzyme activity must also have another cause. The decrease in enzyme specific activity but not protein content may indicate some direct inhibitory effects by estradiol. Recently, Fencl and Villee[31] reported no decrease in 4-ene-5α-reductase activity after 3 days of estradiol administration, whereas a decrease was observed 3 days following castration. They postulate that the mechanisms for prostate involution are different following estradiol administration and castration.

Testosterone administration resulted in increases in prostatic 4-ene-5α-reductase activity in all groups studied. This is expected since the prostate depends on testosterone for its growth and function. But the large increases of the testosterone 4-ene-5α-reductase specific activity in castrated (Fig. 4) and hypophysectomized (Fig. 6) animals suggest that testosterone may have a selective effect in restoring this particular protein. Liao and Fang[32] and Liao *et al.*[33] have demonstrated that following testosterone administration, there is a rapid increase in RNA polymerase activity and the synthesis of nucleolar RNA, ribosomal RNA and ribonucleoprotein particles rich in mRNA. Administra-

tion of testosterone to castrated animals stimulates the incorporation of radioactive amino acids into protein [34]. Recently, Mainwaring and Wilce [35] and Isotalo and Santti[36] also indicated that a rapid synthesis of a few selective species of RNA and proteins may be the primary response of testosterone treatment.

In our experiments, prolactin by itself, had no effect on the prostate either in intact or hypophysectomized animals. In intact rats, endogenous hormones may mask any effects on testosterone 4-ene-5α-reductase activity that the exogenous prolactin could have. In hypophysectomized rats, the prolactin effects may require the presence of a normal functioning adrenal gland [19]. Either of these reasons could give rise to the apparent lack of significant effects of prolactin.

When estradiol, testosterone and prolactin were administered in different combinations, variable results were obtained when testosterone 4-ene-5α-reductase activity was measured. The combination of estradiol and/or prolactin with testosterone produced changes in the enzyme activity which were similar to those found when testosterone alone was given except in the adrenalectomized and castrated groups receiving estradiol and testosterone. Although others [20, 30] have reported that prolactin synergizes with testosterone in its effects on the prostate, our experiments have not shown such action. We have no good explanation for the testosterone-estradiol synergism in the castrated and antagonism in the adrenalectomized animals. Prolactin administration alone had no effect and in combination with estradiol did not significantly alter the estrogen effect on 4-ene-5α-reductase activity.

In summary, our studies here have shown that while *in vivo*, estrogen inhibits and testosterone promotes the growth of the prostate gland, their actions are influenced by other hormones such as those of the adrenal gland.

*Acknowledgements*—This work was supported in part by grants from the National Cancer Institute of Canada and the Ontario Cancer Treatment and Research Foundation (grant 174).

## REFERENCES

1. Dorfman R. I. and Shipley R. A.: In *Androgens: Biochemistry, Physiology and Clinical Significance*. John Wiley and Sons Inc., New York (1956) p. 118.
2. Farnsworth W. E. and Brown J. R.: *J. Am. med. Ass.* **183** (1963a) 426–439.
3. Farnsworth W. E. and Brown J. R.: *Natl. Cancer Inst. Monograph* **12** (1963b) 323–329.
4. Bruchovsky N. and Wilson J. D.: *J. biol. Chem.* **243** (1969) 2012–2021.
5. Anderson K. M. and Liao S.: *Nature* **219** (1968) 277–279.
6. Tveter K. H. and Aakvaag A.: *Endocrinology* **85** (1969) 683–689.
7. Siiteri P. K. and Wilson J. D.: *J. clin. Invest.* **49** (1970) 1737–1745.
8. Giorgi E. P., Stewart J. C., Grant J. K. and Scott R.: *Biochem. J.* **123** (1971) 41–55.
9. Alder A., Burger H., Davis J., Dulmanis A., Hudson B., Sarfaty G. and Straffon W.: *Brit. med. J.* **1** (1968) 28–30.
10. Bird C. E., Green R. N. and Clark A. F.: *Clin. Endocr.* **29** (1969) 123–126.
11. Bird C. E., Green R. N., Calandra R. S., Connolly J. G. and Clark A. F.: *Acta endocr., Copenh.* **67** (1971) 733–739.
12. Bird C. E. and Clark A. F.: *J. clin. Endocr. Metab.* **34** (1972) 467–472.
13. Farnsworth W. E.: *Invest. Urol.* **6** (1968–1969) 423–427.
14. Groom M., Harper M. E., Fahmy A. R., Pierrepoint C. G. and Griffiths K.: *Biochem. J.* **122** (1971) 125–128.
15. Leav I., Morfin R. F., Ofner P., Cavazos L. F. and Leeds E. B.: *Endocrinology* **89** (1971) 465–483.
16. Giorgi E. P., Stewart J. C., Grant J. K. and Shirley I. M.: *Biochem. J.* **126** (1972) 107–121.
17. Lee D. K. H., Young J., Tamura Y., Patterson D. C., Bird C. E. and Clark A. F.: *Can. J. Biochem.* **51** (1973) 735–740.
18. Tissell L. D. and Angervell L.: *Acta endocr., Copenh.* **62** (1969) 694–710.
19. Tullner W. W.: *Natl. Cancer Inst. Monograph* **12** (1963) 211–233.
20. Grayhack J. T.: *Natl. Cancer Inst. Monograph* **12** (1963) 189–199.
21. Delin S., Squire P. G. and Porath J.: *Biochim. biophys. Acta* **89** (1964) 398–408.
22. Maggio R., Siekevitz P. and Palade G. E.: *J. Cell Biol.* **18** (1963) 267–291.
23. Miller G. L.: *Analyt. Chem.* **31** (1959) 964.
24. Lowry O. H., Roseborough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
25. Munro H. N. and Fleck A.; In *Methods of Biochemical Analysis* (Edited by Glick D.). Interscience **14** (1966) 113.
26. Burton K.: *Biochem. J.* **62** (1956) 315–323.
27. Harper M. E., Fahmy A. R., Pierrepoint C. G. and Griffiths K.: *Steroids* **15** (1970) 89–103.
28. Davies P., Fahmy A. R., Pierrepoint C. G. and Griffiths K.: *Biochem. J.* **129** (1972) 1167–1169.
29. Moore R. J. and Wilson J. D.: *Endocrinology* **93** (1973) 581–592.
30. Moger W. H. and Geschwind I. I.: *Proc. Soc. exp. Biol. Med.* **141** (1972) 1017–1021.
31. Fencl M. deM. and Villee C. A.: *Steroids* **21** (1973) 537–552.
32. Liao S. and Fang S.: *Vit. Horm.* **27** (1969) 17–90.
33. Liao S., Barton R. W. and Lin A. H.: *Proc. natn. Acad. Sci., U.S.A.* **55** (1966) 1593–1600.
34. Williams-Ashman H. G., Liao S., Hancock R. L., Jurkowitz L. and Silverman D. A.: *Rec. Prog. Horm. Res.* **20** (1964) 247–301.
35. Mainwaring W. I. P. and Wilce P. A.: *Biochem. J.* **130** (1972) 189–197.
36. Isotalo A. and Santti R. S.: *Biochim. biophys. Acta* **277** (1972) 595–605.