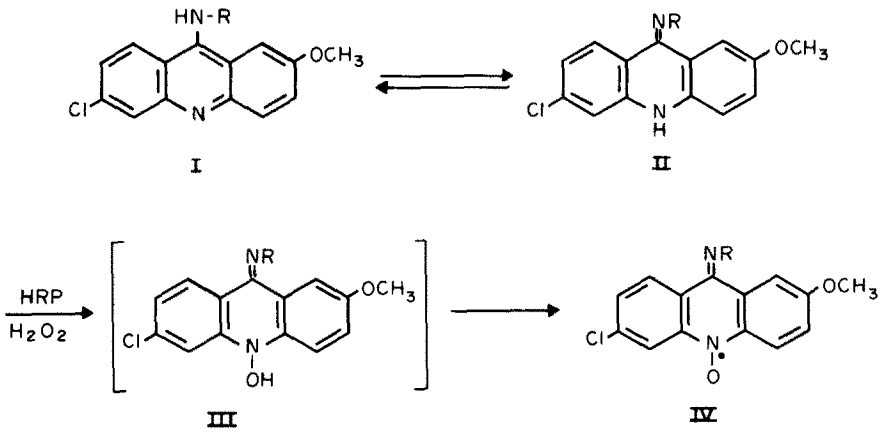

Scheme 1. Proposed formation of quinacrine nitroxide radical by enzymatic activation.

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Androgen dependence of glutathione metabolism in ventral prostate

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A relationship seems to exist between glutathione (GSH) metabolism and cellular proliferation [1]. Relatively high concentrations of glutathione and other sulfhydryl (SH) group containing compounds are required for mitosis and cell division in plants, animals and micro-organisms [2]. Studies on sulfhydryl metabolism during cell division suggest that glutathione might be subject to modulation during carcinogenesis [3]. Hosoda and Nakamura [4] showed that, in Ehrlich ascites tumor cells, the activity of the hexose monophosphate pathway, which supports the supply of materials for DNA and RNA synthesis, is largely dependent upon the availability of NADP, generated from the glu-

tathione redox cycle. Taniguchi *et al.* [5] reported lower levels of glutathione in some primary hepatomas and also in transferable ascites hepatomas in comparison to normal tissues. Evidence thus indicates that glutathione may be involved in both normal and abnormal cell growth [6, 7]. Estrogens have been found to influence glutathione metabolism in uterus and mammary tumors [8]. Glutathione levels are also known to be affected by adrenal, thyroid, pituitary, pancreatic, parathyroid and growth hormones [9, 10].

It is well known that growth, differentiation, and secretory functions of the prostate are androgen dependent. Attempts have been made to elucidate the mechanism of androgen action through a complex progression of molecular reactions [11]. A relationship further seems to exist between androgen action, cyclic AMP and polyamine metabolism [12]. However, at present, information regarding the control of hormonal regulation of glutathione metabolism in the prostate gland is lacking. It was therefore of interest to investigate the interaction between androgens and glutathione metabolism in this accessory sex organ. Our data on the effects of androgen depletion and replacement therapy, as well as on estrogen and antiandrogen-induced changes, suggest that the concentration of glutathione in the ventral prostate is a function of the androgenic status.

Materials and methods

Adult male Fischer 344 rats, weighing 200–225 g, maintained on Master Laboratory Chow and water *ad lib.*, were used in this study. Testosterone (50 mg/kg), β-estradiol (2 mg/kg) and cyproterone acetate (12.5 mg/kg) were injected intramuscularly daily for 7 days to groups of normal animals. The control group received an equal volume of corn oil as the vehicle. Animals were killed 24 hr after the last injection by cervical dislocation and the ventral prostate were rapidly excised, freed from extraneous tissue, and frozen in liquid nitrogen. The tissue was kept frozen at −60° until the time of analysis.

Castration was performed under sodium pentobarbital anesthesia (40 mg/kg i.p.). Following 7 days of castration, testosterone (50 mg/kg) or β-estradiol (2 mg/kg) was injected daily for another 7 days. Animals were killed after 3 and 7 days of hormone administration, and the prostates were excised and processed as described below.

Portions of the frozen tissue were homogenized in a polytron homogenizer with phosphate buffer (0.1 M) containing 0.005 M EDTA, pH 8.0, to yield a final concentration of 5%. Reduced glutathione (GSH) and oxidised glutathione (GSSG) were estimated by following the method of Cohn and Lyle [13], as modified by Hissin and Hilf [14] using *O*-phthalaldehyde as the fluorescent agent and measuring fluorescence at 420 nm with the activation at 350 nm in an Aminco Bowman spectrofluorometer. Standard curves were constructed for the assay of reduced and oxidised glutathione, and the values are expressed as μg GSH or GSSG per mg protein. Each estimation of glutathione was made in ventral prostate pooled from three rats. Protein was determined by the method of Lowry *et al.* [15], with bovine serum albumin as the standard.

Testosterone, β-estradiol, GSH, GSSG and *O*-phthalaldehyde were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Cyproterone acetate was supplied by Schering A.G., Berlin, West Germany.

Results

Effects of testosterone, estradiol and cyproterone acetate on prostatic glutathione in normal rats. The effects of androgen, estrogen and antiandrogen on prostate weights and glutathione concentration following treatment for 7 days were investigated (Table 1). Administration of testosterone produced a slight increase in the prostate weight of normal animals. Cyproterone acetate reduced organ weight to 19% of the normal values. Estradiol treatment also produced a significant decrease (33%) in prostate weight. The levels of reduced glutathione also were lowered significantly following treatment with estradiol or cyproterone acetate. In contrast, the concentration of oxidised glutathione was not altered to any appreciable extent by either treatment. As a result, the GSH/GSSG ratio was reduced significantly by estradiol and cyproterone acetate. The simultaneous administration of testosterone and cyproterone acetate did not produce any significant change in weight, protein content and oxidised or reduced glutathione levels in the ventral prostate.

Effect of castration and testosterone replacement therapy

Table 1. Effects of testosterone, estradiol and cyproterone acetate treatment on glutathione content of ventral prostates in normal rats*

Treatment	Prostate weight (mg)	Protein (mg/100 mg)	GSH (μg/mg protein)	GSSG (μg/mg protein)	GSH/ GSSG
Control	267.0 ± 14.7 (100)	9.6 ± 1.3 (100)	2.08 ± 0.1 (100)	0.50 ± 0.07 (100)	4.17 ± 0.1 (100)
Testosterone (50 mg/kg)	320.0 ± 20.0 (119)	11.0 ± 0.8 (115)	2.10 ± 0.24 (100)	0.45 ± 0.03 (90)	4.6 ± 0.18 (110)
β-Estradiol (2 mg/kg)	180.0 ± 16.2 (67)†	7.2 ± 0.8 (75)†	1.49 ± 0.5 (72)†	0.45 ± 0.03 (90)	3.3 ± 0.08 (78)†
Cyproterone acetate (12.5 mg/kg)	53.0 ± 5.6 (19)†	7.0 ± 1.3 (77)†	1.28 ± 0.09 (62)†	0.50 ± 0.05 (100)	2.5 ± 0.07 (59)†
Testosterone (50 mg) + cyproterone acetate (12.5 mg)	312.0 ± 22.0 (116)	10.1 ± 22.0 (105)	2.11 ± 0.22 (101)	0.45 ± 0.03 (90)	4.6 ± 0.1 (110)

* Each value represents the mean ± S.E.M. of three pooled samples of ventral prostate. For each experimental group, prostates from three rats were pooled for one sample, and three such samples were then used for each assay. The rats were treated daily by the i.m. route, either with testosterone, estradiol or cyproterone acetate for 7 days. Controls were injected with an equal volume of the vehicle alone (corn oil). Animals were killed 24 hr after the last injection. Data in parenthesis express results in percentages, taking the values of control rats as 100%.

† Statistically significant difference when compared with the values of control rats ($P < 0.05$).

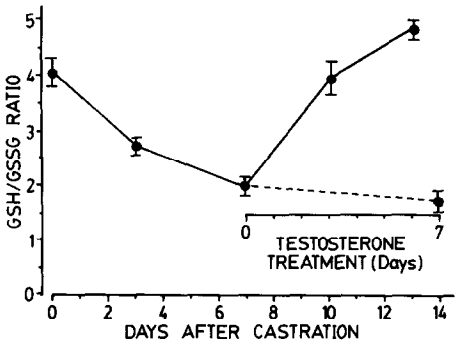

Fig. 1. Effects of castration and subsequent administration of testosterone (50 mg/kg) on the GSH/GSSG ratio of rat ventral prostate. Each point represents the mean ± S.E.M. of three separate determinations. Pooled material from three animals was used for each assay (see legend to Table 1). The GSH/GSSG ratio decreased to 65, 47 and 40% of normal values taken as 100% following 3, 7 and 14 days castration respectively. Testosterone treatment restored the ratio to 93 and 118%, respectively, at 3 and 7 days of hormone administration. Key: (– – –) changes in prostate of castrated animals that were not injected with testosterone.

on prostatic glutathione. Gonadectomy for 3 days resulted in a considerable atrophy of the ventral prostate as evidenced by a 55% reduction in its weight which was more pronounced (68%) at 7 days of castration (Fig. 1). Administration of testosterone to gonadectomized animals daily for 7 days restored organ weights to normal limits. The GSH/GSSG ratio was reduced significantly in prostate of castrated animals and returned to normal values with testosterone replacement. Since there was little or no effect on oxidised glutathione after castration and testosterone treatment, the observed change in the GSH/GSSG ratio was due to an effect on reduced glutathione alone. Estrogen treatment did not produce any significant change in the concentration of reduced or oxidised glutathione in ventral prostates of castrated animals (data not shown).

Discussion

Since the growth and the function of normal prostate are androgen-dependent processes coupled with the emerging role of glutathione during carcinogenesis [8], studies of the influence of androgens on glutathione metabolism are of obvious current interest. Experiments were undertaken, therefore, to examine the effects of gonadectomy and of androgen, antiandrogen and estrogen treatment on glutathione levels of rat ventral prostate.

Gonadectomy resulted in a significant decrease in GSH which was restored to normal values following testosterone treatment. Administration of an estrogen or antiandrogen to normal rats produced a significant reduction in GSH levels. In contrast, estrogen treatment in gonadectomized animals failed to exert any appreciable effect. This lowering effect of estradiol on GSH of intact, but not castrated, animals suggests that estrogens may be acting indirectly on glutathione metabolism of ventral prostate. It is known that estrogens also can act indirectly via the inhibition of gonadotropin secretion in suppressing testicular androgen synthesis [16].

Cyproterone acetate, an androgen antagonist, exerts its inhibitory effects on nuclear activity of ventral prostates including the synthesis of RNA [17], DNA [18], and protein [19]. Our present data on the influence of cyproterone acetate suggest that at least some of its metabolic consequences may involve modulation of glutathione metabolism since sulfhydryl groups are known to be associated in the synthesis of these macromolecules.

The observed decrease in prostatic glutathione by cyproterone acetate was antagonized by simultaneous administration of testosterone. The concentration of oxidized glutathione was not affected by any of the treatments employed.

Glutathione carries out the majority of its functions in the reduced form, the concentration of which is regulated by the enzyme glutathione reductase. In this regard, it may be noted that the activity of glutathione reductase was reduced significantly (unpublished data) after gonadectomy. The observed decrease in GSH level with no appreciable effect on the oxidised form could most likely be due to a decline in enzyme activity produced by gonadectomy. The present data demonstrate that the maintenance of normal glutathione levels in the ventral prostate is contingent upon the androgenic status of the animal. Furthermore, androgen regulation of glutathione metabolism during normal and abnormal growth of the prostate needs to be elucidated further as significant changes in glutathione have been reported to occur in chemically induced hepatomas and fetal rat liver [5]. The recent report [20] that the administration of GSH caused regression of tumor growth further attests to the importance of studying glutathione metabolism in carcinoma of the prostate.

In summary, our data demonstrate that glutathione metabolism in ventral prostate is subject to modulation by androgenic influences. Whereas castration decreased the concentration of GSH, it was restored to normal limits following treatment with testosterone. Cyproterone acetate was an androgen antagonist with regard to the prostatic glutathione metabolism as well. Estradiol affects glutathione levels, perhaps indirectly, by inhibiting gonadotropin release, thereby suppressing androgen synthesis in the ventral prostate.

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Induction of hepatic microsomal cytochrome P-450 and associated monooxygenases by pentamethylbenzene in the rat

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Cytochrome P-450, the terminal oxidase of the hepatic microsomal election transport system, and a variety of mixed-function oxidase (MFO) activities are inducible following treatment of animals with a wide variety of zenobiotics [1–3]. Multiple forms of mammalian cytochrome P-450 have also been identified and characterized by enzymatic, immunological, and biochemical techniques [2–6].

Insects, like mammals, have been shown to possess inducible MFO systems [7–9], and studies in this laboratory with the southern armyworm (*Spodoptera eridania*) have established that MFO activity in the larval midgut tissues of this insect is highly responsive to dietary treatment with pentamethylbenzene (PMB) and other alkylbenzenes [8]. Subsequent studies have shown that, of the several non-insect species tested, only MFO activities of the rat were affected appreciably by PMB administration. This communication reports some of the unusual effects of PMB administration on rat hepatic MFO activities.

Male Sprague–Dawley-derived rats (200 mg), obtained from Blue Spruce Farms, Altamont, NY, were injected i.p. for 3 successive days with either phenobarbital (PB, 100 mg/kg) in 0.9% saline), 3-methylcholanthrene (MC, 20 mg/kg in corn oil), or pentamethylbenzene (PMB, 2.5 g/kg in corn oil). Control animals received the appropriate vehicles only, and no significant differences were observed between MFO values in microsomes from control and untreated rats. Animals (four per treatment group) used in the dose–response study received a single injection of the appropriate concentration of each inducer, and microsomes were prepared 24 hr later. In all cases, microsomes were prepared as previously described [10] and protein was determined by the method of Lowry *et al.* [11], using bovine serum albumin as a standard. Cytochrome P-450 was measured according to the method of Omura and Sato [12] using an extinction coefficient of 91 mM^{-1} cm^{-1} for the CO-ferrocytochrome P-450 spectral complex. Aminopyrene *N*-demethylase (APDM) activity was measured by the liberation of formaldehyde [13], and aniline *p*-hydroxylase (APH) activity was assayed as previously described [14]. For APDM and APH activities, incubation contained cofactors as described previously and consisted of 16 μmoles of glucose-6-phosphate (G-6-P), 2 units of G-6-P dehydrogenase and 2.5 μmoles of NADP. Aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH) activity was measured by the direct fluorometric assay of Yang and Kicha [15], and 7-ethoxycoumarin *O*-deethylase (7EC) activity was determined according to the method of Ullrich and Weber [16]. The inhibitors α-naphthoflavone (ANF) and 1-phenylimidazole (PI) were employed *in vitro* at final concentrations of 10^{-5} M and 10^{-4} M respectively.

Benzo[*a*]pyrene, PMB and ANF were purchased from the Aldrich Chemical Co., Milwaukee, WI. PB, MC and NADPH were from the Sigma Chemical Co., St. Louis, MO; G-6-P, G-6-P dehydrogenase and NADP were from Boehringer Mannheim Biochemicals, Indianapolis, IN; and PI was obtained from Trans World Chemicals, Washington, DC. All other chemicals were of analytical reagent grade.

The effects of pretreating rats with PMB were compared with those resulting from PB- and MC-treatment with respect to hepatic microsomal cytochrome P-450 levels and associated oxidase activities. Dose–response relationships from a single administration of the inducer, as well as the effects of repeated dosing, were studied.

Dose–response curves for the effects of PB, MC and PMB of 7EC and AHH activities (Fig. 1) clearly showed that PMB was not an effective inducer of AHH activity.

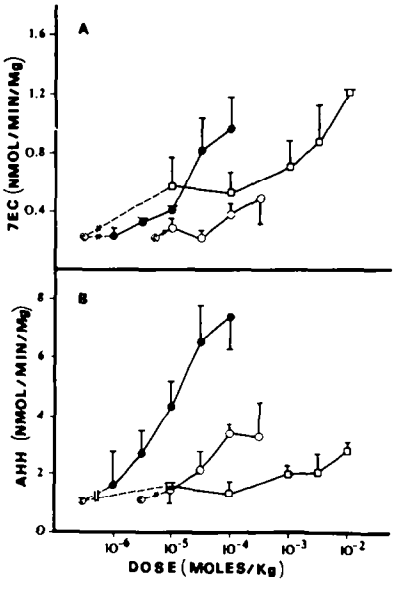

Fig. 1. Dose-response curves for the induction of 7EC (A) and AHH (B) activity in the rat. Enzyme assays and treatments with vehicle alone (C), MC (●——●), PB (○——○), and PMB (□——□) were described in the text. Each value represents the mean ±S.D. of at least four animals.