Effect of Androgen on Glucose-6-Phosphate Dehydrogenase Isoenzymes in Rat Ventral Prostate and Seminal Vesicles[1] (37530)

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It seems well established that glucose-6-phosphate dehydrogenase (G6PD) exists in multiple molecular forms or isoenzymes. The isoenzyme pattern has been extensively studied in red blood cells (1) and numerous isoenzymes were reported to exist in liver (2, 3), in the mammary gland (4, 5), in certain mammary carcinomas (6, 7) and in the uterus (8). In a recent study, Hilf *et al.* (8) demonstrated that the dose-related increase in uterine G6PD activity elicited by estradiol benzoate was primarily due to the response of one discrete G6PD isoenzyme. The specificity of this response was demonstrated by the ability of the anti-estrogen, U11100A, to prevent the estrogen-induced elevation in isoenzyme activity. Similarly, the G6PD isoenzyme response to estrogens in the mammary gland and in two different mammary adenocarcinomas was limited to discrete isoenzyme species (5–7).

Both the ventral prostate and seminal vesicles of the rat are androgen target organs and Lerner *et al.* (9) demonstrated that administration of testosterone propionate caused a dose-related increase in total G6PD activity in the male accessory sex organs. The simultaneous administration of an anti-androgen, A-norprogesterone, prevented the androgen-induced response in G6PD; administration of the anti-estrogen, MER-25, did not interfere with the responses elicited by testosterone propionate in these target tissues. The increase in G6PD activity in the male accessory sex organs after treatment with androgen can be prevented by concomitant administration of actinomycin D or by cycloheximide, results which led Singhal and Ling (10) to propose that the response in G6PD was a result of enzyme synthesis *de novo*.

It seemed logical, therefore, to ascertain the patterns of G6PD isoenzymes in the ventral prostate and seminal vesicles and to investigate the effects of androgen treatment on these isoenzymes. The data presented here show that each of the accessory sex organs of the male possessed a distinct G6PD isoenzyme profile, which can be discretely altered by administration of testosterone propionate.

Materials and Methods. Animals and treatment. Thirty-five-day-old male Fischer rats, weighing 60–80 g, were used throughout the study. Castration was performed under ether anesthesia by cutting the scrotal sac between the testes and making two more cuts through tunica albigans. The testes were individually isolated and excised after the spermatic cord was ligated. The incision was closed using wound clips and the animals were allowed to recover from surgery for 7 days. Solutions of testosterone propionate (Schering A. G. Berlin) were made with sesame oil. Various doses were administered subcutaneously in a volume of 0.1 ml.

Tissue preparation. All animals were killed by cervical dislocation after 8 days of hormone therapy. Seminal vesicles and ventral prostates were quickly excised, trimmed of

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adhering fat and weighed. The seminal vesicles were lightly blotted with gauze but no effort was made to completely express the vesicular fluid. Homogenates, 33% (w/v), were rapidly prepared in cold 0.05 *M* Tris buffer, pH 7.4, using a mechanically driven Duall tissue grinder. Cytosols were obtained after centrifugation at 20,000*g* at 4° for 4 min. An aliquot of the supernatant was taken for protein assay by the method of Lowry *et al.* (11).

Enzyme assay. An aliquot of the supernatant was taken for the assay of G6PD (glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49), according to the method of Glock and McLean (12). Assays were performed on a Gilford Model 2400 spectrophotometer with a constant temperature cuvette set at 25°. Optical density changes were measured at 340 nm for the production of NADPH and reaction conditions were such that zero order kinetics were obtained for at least 5 min. The average change in absorbance per minute was determined and converted to micromoles of NADPH formed per minute (U)/100 mg of tissue or per milligram protein.

Gel electrophoresis. Acrylamide disc gel electrophoresis was performed by a technique similar to that described by Dietz and Lubrano (13) and Dietz *et al.* (14). A Model 1200 electrophoresis bath made by Canal Industrial Corp., Rockville, Md. and acrylamide and *N,N'*-methylenebisacrylamide, (enzyme grade products, Eastman Kodak Co.) were used during the study.

A solution containing acrylamide, *N,N'*-methylenebisacrylamide, Tris buffer, and ammonium persulfate, producing a final gel concentration of 5.5%, was pipetted into 7 mm i.d., 7 cm long, detergent treated glass tubes to a depth of 44 mm. Water was carefully layered over the top of the gels and they were polymerized by exposure to a 15 W fluorescent light for 30 min. After the water was removed, 15 μl of the cytosol, mixed with 50 μl of 40% sucrose, was layered on the gel. The sample was then overlaid with 10 mm of a 5.5% cap gel, prepared as above and again polymerized with light. All samples were processed at least in triplicate. Electrophoresis was performed at 4°, using Tris–glycine buffer, pH 7.3, with 2.5 mA per gel, for the amount of time it took the tracking dye (bromphenol blue) to travel the length of the gel (approximately one hour). The gels were removed from the tubes and incubated in the dark for localization of the G6PD isoenzymes as detailed by Richards and Hilf (5). After incubation for 1–2 hr at 37°, the gels were washed with distilled water and stored in 7% acetic acid to terminate the enzymatic reaction.

The gels were scanned at 600 nm in a linear transport device attached to the Gilford spectrophotometer, using a scanning rate of 1 cm/min, a slit setting of 0.05 mm, and a scale range of 0–0.5 absorbance units. The densitometric tracing produced symmetrical peaks, enabling us to calculate the relative percentage of each isoenzyme after measuring the areas of each peak by triangulation (15). A linear relationship has been demonstrated between the area under the peaks and the amount of time for the enzyme reaction as well as for the level of activity. Isoenzyme species were estimated by multiplying the total activity placed on the gel by the percentage of each stained band.

All data are presented as the mean ± standard error of the mean.

Results. Table I summarizes the data on the weights and total G6PD activity of the seminal vesicles and ventral prostates from castrated rats that had received various doses of testosterone propionate. A daily dose of 0.008 mg of testosterone propionate produced a doubling in the weights of the accessory sex organs; higher doses of the androgen produced further increases in seminal vesicles and ventral prostate weight. The activity of G6PD in the accessory sex organs of castrate rats receiving daily doses of 0.040 mg of testosterone propionate was approximately equal to the G6PD activity found in the accessory sex organs of the intact rat of similar age.

The activity of G6PD/100 mg seminal vesicles or ventral prostate was doubled in animals that received 0.008 mg testosterone propionate per day. The activity of the enzyme showed further increases with increas-

TABLE I. Effect of Daily Administration of Testosterone Propionate on Weight and Glucose-6-Phosphate Dehydrogenase Activity in Ventral Prostate and Seminal Vesicles.

Treatment[a]	Daily dose (mg)	Ventral prostate Wt (mg/100 g)	Ventral prostate G6PD (U/100 mg)	Ventral prostate G6PD (U/mg prot)	Seminal vesicles Wt (mg/100 mg)	Seminal vesicles G6PD (U/100 mg)	Seminal vesicles G6PD (U/mg prot)
Control-intact	0	53 ± 4[b]	0.159 ± 0.020	0.015 ± 0.001	106 ± 4	0.081 ± 0.009	0.009 ± 0.001
Control-castrate	0	8 ± 1	0.052 ± 0.012	0.011 ± 0.001	11 ± 1	0.033 ± 0.012	0.006 ± 0.002
Castrate-test. prop.	0.008	14 ± 1	0.098 ± 0.002	0.016 ± 0.001	20 ± 1	0.065 ± 0.011	0.009 ± 0.002
Castrate-test. prop.	0.040	46 ± 2	0.152 ± 0.011	0.017 ± 0.001	90 ± 4	0.080 ± 0.006	0.009 ± 0.001
Castrate-test. prop.	0.200	75 ± 4	0.186 ± 0.013	0.021 ± 0.002	175 ± 7	0.103 ± 0.018	0.013 ± 0.002
Castrate-test. prop.	1.000	94 ± 4	0.167 ± 0.024	0.017 ± 0.003	228 ± 8	0.108 ± 0.015	0.015 ± 0.001

[a] Each treatment group consisted of 6 animals.
[b] Mean ± SEM.

ing androgen doses; the peak activity occurred in animals that received 0.20 mg testosterone propionate daily. Specific G6PD activity (μmoles NADPH produced/min/mg protein) was significantly elevated in the seminal vesicles and ventral prostates of rats that received as little as 0.008 mg testosterone propionate daily. Further increases in G6PD activity resulted from increasing doses of hormone. The maximum specific activity occurred in seminal vesicles of animals that received 1.0 mg testosterone propionate daily, while maximum specific activity in the ventral prostate was elicited by 0.20 mg testosterone propionate.

Four well-defined isoenzymes of G6PD were observed by disc gel electrophoresis (Fig. 1), and were numbered on the basis of order of decreasing mobility under the conditions employed here. As shown in Fig. 2, the predominant isoenzyme was G6PD-1 in the ventral prostate of untreated, noncastrated rats, whereas the predominant isoenzyme was

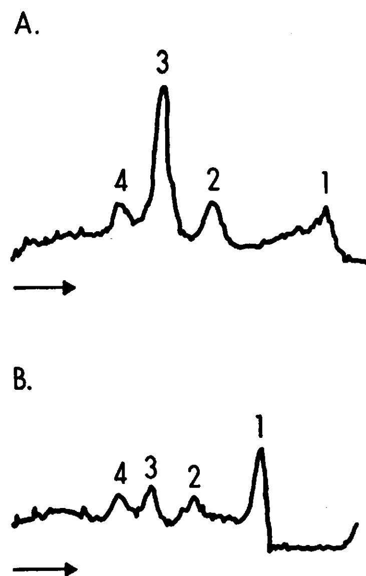

FIG. 1. Representative tracings from gels stained for G6PD activity and scanned at 600 nm (See Materials and Methods). Samples obtained from castrate rats that received 1.00 mg testosterone proprionate per day × 8. The discrete peaks representing 4 isoenzymes are numbered in descending order of mobility. Arrow shows direction of migration. A, seminal vesicles; B, ventral prostate.

G6PD-3 in the seminal vesicles of these animals.

In castrated, diluent-injected animals, G6 PD-3 was the predominant isoenzyme in both the seminal vesicles, where it accounted for 50% of the total activity, and in the ventral prostate, where it accounted for 80% of the total activity. In the ventral prostate, the dose-related increase in G6PD activity after administration of testosterone propionate was primarily due to an increase in the activity of G6PD-1, the maximum increase being a 23-fold elevation above the untreated animals. There was also observed a slight decrease in

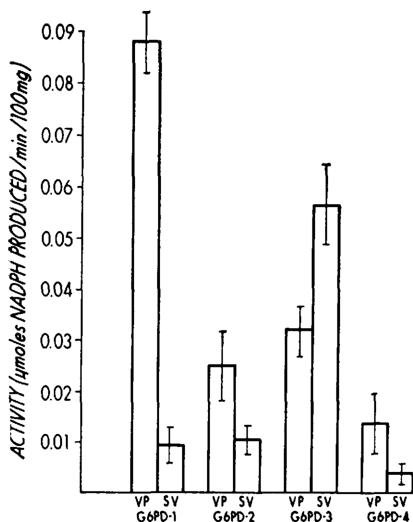

FIG. 2. Estimated G6PD isoenzyme activity in young adult (50 day old) intact Fischer rats in ventral prostate (VP) and seminal vesiclie (SV).

the activity of G6PD-2 and a slight increase in the activity of G6PD-3 in prostates of androgen-treated animals when compared with the prostates from diluent-injected control animals (Fig. 3). In the seminal vesicles, the increase in G6PD activity elicited by androgen was primarily due to a 3- to 4-fold increase in G6PD-3 activity and a 2- to 3-fold increase in G6PD-1 (Fig. 4). Treatment with androgen did not alter the activities of G6 PD-2 and G6PD-4 of the seminal vesicles. Thus, the administration of testosterone propionate to castrate rats produced a different response in the G6PD isoenzymes in the ven-

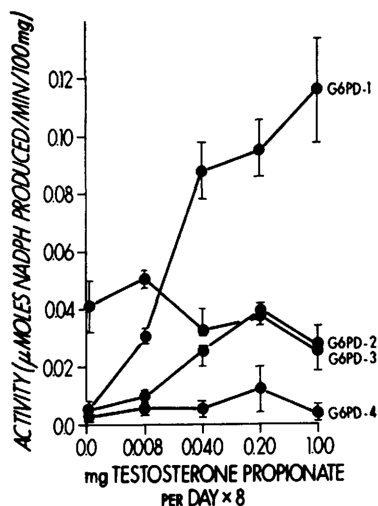

FIG. 3. Effect of various doses of testosterone propionate on G6PD isoenzyme activities in ventral prostate from castrate rats. Each point is the mean of 6–8 animals and vertical lines represent SEM.

tral prostate as compared with the response observed in the seminal vesicles.

Discussion. The data presented here indicate that G6PD exists as four well-defined isoenzymes in both seminal vesicles and ventral prostate, as demonstrated by electrophoretic separation on polyacrylamine gels. Al-

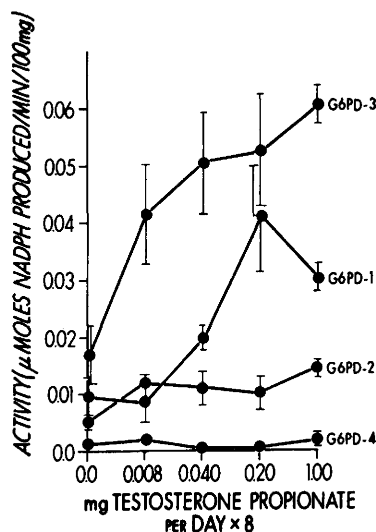

FIG. 4. Effect of various doses of testosterone propionate on G6PD isoenzyme activities in seminal vesicles from castrate rats. Each point is the mean of 6–8 animals and vertical lines represent SEM.

though both ventral prostate and seminal vesicles are androgen-dependent and androgen-responsive end organs, they demonstrate different isoenzyme patterns in the presence of endogenous androgens with G6PD-1 representing the predominant isoenzyme species in ventral prostate and G6PD-3 representing the predominant species in seminal vesicles. Similarly, the G6PD-1 isoenzyme was the most responsive species in the ventral prostate to administration of exogenous testosterone propionate whereas G6PD-3 was the most responsive species in the seminal vesicles to treatment with androgen (although G6PD-1 in the seminal vesicles was also elevated after injection of testosterone propionate). It is of interest that the isoenzyme patterns of both of these accessory sex organs were quite similar after removal of endogenous androgen by orchiectomy (castrate control).

There have been several reports indicating the presence of G6PD in mitochondria (16, 17) and in microsomes (18). Although these investigators only examined liver, it is quite possible that G6PD may also be localized in these subcellular fractions in the male accessory sex organs. The techniques used would not prevent their entry into the cytoplasmic fraction studied here; study of such subcellular fractions is currently underway.

Summary. The seminal vesicles and ventral prostate of Fischer rats were found to contain 4 discrete glucose-6-phosphate dehydrogenase (G6PD) isoenzymes as estimated by separation on disc gel polyacrylamide electrophoresis followed by specific staining and planimetry. The activities (μmole NAD PH/min/100 mg tissue) in intact young adult rats for each G6PD isoenzyme were: seminal vesicles; G6PD-1, 0.009; G6PD-2, 0.011; G6PD-3, 0.057; and G6PD-4, 0.004 (numbering based on order of decreasing mobility); ventral prostate; G6PD-1, 0.088; G6 PD-2, 0.025; G6PD-3, 0.032; and G6PD-4, 0.014. Thus, the predominant isoenzyme in ventral prostate was G6PD-1, while in the seminal vesicles G6PD-3 was the predominant isoenzyme.

In rats sacrificed 2 weeks after castration, G6PD-3 was the predominant isoenzyme in both seminal vesicles and ventral prostate. Testosterone propionate, administered subcutaneously at 8, 40, 200, or 1000 μg/day/animal produced a dose-related response in total G6PD activity. In the ventral prostate this increase was primarily due to a dose-related increase in G6PD-1 (maximum increase was 23-fold), a slight decrease in G6PD-2 and a slight increase in G6PD-3 when compared to the diluent-injected castrate control animals. In the seminal vesicles, the increase in G6PD activity was primarily due to a 4-fold increase in G6PD-3 and a 2- to 3-fold increase in G6PD-1. Thus, elevation of G6 PD activity by androgen treatment appears to be the result of induction of different isoenzyme species in seminal vesicles and in ventral prostate.

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