# Testicular Metabolism in Adrenalectomized and Corticosterone Treated Rats[1] (35625)

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Treatment of adult rats with adrenocorticotropic hormone (ACTH) reduces testis weight and causes Leydig cell atrophy (1, 2). Similarly, testicular and accessory sex gland involution have been associated with increased adrenocortical activity in adult rodents exposed to high population densities and to low environmental temperatures (3, 4). Despite evidence linking increases in plasma corticosteroids with testis dysfunction numerous experiments have failed to alter the spermatogenic and steroidogenic functions of the testis by administering cortisone, or one of its derivatives (5). On the other hand, a few reports indicate that cortisone exerts a deleterious effect on the germinal epithelium of rats and mice (5). In view of the ambiguity of previous studies and the fact that cortisone is not the predominant glucocorticoid in laboratory rodents (6) the present study was initiated to examine the effects of adrenalectomy and corticosterone replacement on testis metabolism in adult rats. Testicular metabolism was used to assess the effects of adrenalectomy and corticosterone on the testis because it was anticipated that metabolic responses could provide an index of the influence of corticosteroids that could not be detected by examining alterations in the weight and morphology of the testis.

The effects of adrenalectomy and corticosterone replacement were evaluated by: (i) measuring the concentration of some testicular biochemical components; (ii) examining the capacity of the testis to catabolize glucose *in vitro*; and (iii) determining the amount of testosterone synthetized from acetate-1-$^{14}$C *in vitro*. The results suggest that adrenalectomy may enhance testicular glucose catabolism and that corticosterone (3 mg/day) may interfere with testis metabolism by depressing glucose utilization and testosterone synthesis.

*Materials and Methods.* Mature (300 ± 5 g) Sprague Dawley rats (Cheek Jones, Inc., Houston, Texas) were housed individually in a light (14 hr light and 10 hr darkness/24 hr) and temperature (22 ± 1°) controlled room. Feed (Purina Laboratory Chow) and water were provided *ad libitum*; but after adrenalectomy 0.85% sodium chloride was offered in addition to tap water. Bilateral adrenalectomy was performed under ether anesthesia by making incisions on the left and right sides of rats at an angle between the last rib and vertebral column. The experiment was initiated 24 hr after adrenalectomy by selecting 10 rats for each of the following treatments: (i) unoperated controls; (ii) sham adrenalectomized; (iii) adrenalectomized; (iv) adrenalectomized plus 0.75 mg of corticosterone; (v) adrenalectomized plus 1.50 mg of corticosterone; and (vi) adrenalectomized plus 3.0 mg of corticosterone. The amount of corticosterone injected was similar to or two and four times greater than the level of corticosterone required to maintain adrenalectomized rats (7). Corticosterone (11$\beta$,21-dihydroxy-$\Delta^4$-pregnene-3,20-dione) was dissolved in corn oil and injected (subcutaneously) daily for 5 days at the indicated dose. Sham adrenalectomized and adrenalectomized rats were injected with corn oil alone whereas control rats were not injected. The experiment was planned as a

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completely randomized block design with 10 replicates/block except for data pertaining to testosterone biosynthesis where some samples were lost prior to analysis. Results were subjected to analysis of variance and differences between treatment means were tested by within treatment variance to determine the significance of specific treatment means.

Rats were killed 24 hr after the last injection by cervical dislocation. Testes were removed, trimmed of adhering tissue, weighed, and placed in ice-cold 0.154 *M* KCl. Teased testis tubules were prepared (8) in an ice-cold Petri dish containing a few drops of 0.154 *M* KCl and blotted before weighing. Testis tubules (75–100 mg of fresh wt) were placed in 10 ml of 95% ethanol and assayed for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (9). Testis tubules were homogenized (10%; w/v) in 0.154 *M* KCl with an all glass Ten-Broeck homogenizer. Aliquots of the homogenate were used for carbohydrate (10) and protein (11) analysis and for determining anaerobic glucose metabolism by measuring the amount of $CO_2$ evolved from bicarbonate buffer by acid end products during glycolysis (12). Aerobic glucose metabolism was assessed by measuring the amount of $O_2$ required to oxidize succinate by cell free homogenates of testes (12). Oxidation of glucose-1-$^{14}$C and glucose-6-$^{14}$C to $^{14}CO_2$ was determined separately by placing teased testis tubules (200 mg of fresh wt) in ice-cold Warburg flasks with 3 ml of Krebs–Ringer bicarbonate (KRB) buffer (8) (pH 7.4) containing the appropriate isotope of D-glucose-$^{14}$C (0.5 $\mu$Ci; 10 m*M*). Concentrations of glucose present in the incubation flasks were saturating since increased concentrations of this substrate did not result in increased $^{14}$C incorporation into $^{14}CO_2$. Flasks were flushed with 95% $O_2$:5% $CO_2$, stoppered and incubated at 32° for 2 hr with constant shaking. The reaction was terminated by adding 0.25 ml of 5 *N* $H_2SO_4$. Carbon dioxide was trapped in 0.25 ml of hydroxide of Hyamine (Packard Instrument Co., Downers Grove, Ill.) in the center well of the flask by shaking the flasks for 2 hr at room temperature. The contents of the center well were rinsed three times with a total of 1 ml of methanol and the methanol–Hyamine mixture was transferred to a scintillation vial with 12 ml of scintillation fluid (13) for determination of radioactivity. Control flasks ("zero time") were prepared for each replicate by adding acid and Hyamine to two flasks immediately after placing the buffer–isotope mixture in flasks containing testis tubules.

Testosterone biosynthesis was examined by incubating 500 mg of teased testis tubules in a 50-ml Erlenmeyer flask with 5 ml of KRB buffer (pH 7.4) containing glucose (10 m*M*) and sodium acetate-1-$^{14}$C (25 $\mu$Ci; 2.5 m*M*) for 2 hr under 95% $O_2$:5% $CO_2$ at 32°. The levels of sodium acetate placed in the incubation media were saturating since increased concentrations of acetate did not result in increased $^{14}$C incorporation into testosterone-$^{14}$C. The reaction was terminated by homogenizing the tissue in the incubation media and extracting the media three times with 20 vol of dichloromethane. Testosterone was isolated from the combined extracts and quantitated as testosterone monochloroacetate via gas–liquid chromatography (14). The specificity of the procedure was checked by comparing mass spectra for isolated and authentic testosterone monochloroacetate. Testosterone-1-2-$^{3}$H was used to determine the recovery of testosterone-$^{14}$C through the extraction and quantitation procedures. Radioactivity was determined in 12 ml of toluene scintillation solution containing 15.15 g of PPO (2,5-diphenyloxazole) and 151.5 mg of POPOP (1,4-bis-2, 5-phenyloxazole)/3.79 liters of nanograde toluene.

*Results.* Biochemical (Table I) and metabolic responses (Tables II and III) associated with testes from control (undisturbed) and sham adrenalectomized rats were not significantly different ($p>0.25$) indicating that effects attributed to adrenalectomy and/or corticosterone replacement were not confounded with effects of anesthesia, handling, and operative trauma. The average weight of testes from control, adrenalectomized, and corticosterone (0.75, 1.5 and 3.0 mg)-treated rats was not significantly different ($p>0.25$). Testes from control and adrenalectomized rats contained equivalent amounts of protein ($p>0.15$) but the average concentration of

TABLE I. Effect of Adrenalectomy (ADX) and Corticosterone Replacement on the Weight and Biochemical Composition of Rat Testes.

| Criteria[a] | Control | Sham ADX | ADX | Corticosterone-treated ADX rats[b] (mg): 0.75 | 1.5 | 3.0 |
|---|---|---|---|---|---|---|
| Testes wt (g) | 3.67 ±0.05 | 3.64 ±0.05 | 3.46 ±0.07 | 3.50 ±0.05 | 3.46 ±0.07 | 3.52 ±0.08 |
| Soluble protein (mg/g) | 68.38 ±0.78 | 68.16 ±0.88 | 66.03 ±0.77 | 67.27 ±1.00 | 66.22 ±0.97 | 62.05 ±0.87 |
| Total carbohydrate (mg/g) | 1.80 ±0.19 | 1.93 ±0.19 | 1.39 ±0.14 | 1.61 ±0.10 | 1.87 ±0.29 | 1.97 ±0.21 |
| DNA (mg/g) | 3.38 ±0.03 | 3.29 ±0.04 | 3.32 ±0.04 | 3.31 ±0.04 | 3.32 ±0.04 | 3.30 ±0.05 |
| RNA (mg/g) | 5.42 ±0.09 | 5.63 ±0.10 | 5.23 ±0.08 | 5.44 ±0.12 | 5.40 ±0.10 | 5.15 ±0.12 |
| RNA/DNA | 1.60 ±0.04 | 1.71 ±0.03 | 1.57 ±0.04 | 1.64 ±0.04 | 1.63 ±0.03 | 1.56 ±0.03 |

[a] Each value represents the mean ± standard error of 10 rats.

[b] Adrenalectomized rats were injected once every 24 hr for 5 days with either corn oil or the indicated dose of corticosterone and used 24 hr after the last injection.

testicular protein decreased ($p<0.05$) from 68.4 mg/g in control rats to 62.1 mg/g following corticosterone (3.0 mg) replacement. Other levels of corticosterone (0.75 and 1.5 mg/day) did not produce any significant change ($p>0.25$) in testis soluble protein. To-

TABLE II. *In Vitro* Metabolic Responses of Rat Testicular Tissue After Adrenalectomy (ADX) and/or Corticosterone Treatment.

| Metabolic response[a] | Control | Sham ADX | ADX | Corticosterone-treated ADX rats[b] (mg): 0.75 | 1.5 | 3.0 |
|---|---|---|---|---|---|---|
| Glycolytic activity[c] | 77.65 ±1.64 | 76.81 ±1.74 | 87.88 ±2.09 | 74.26 ±1.29 | 75.70 ±1.21 | 68.06 ±1.32 |
| Krebs' cycle activity[d] | 21.33 ±1.05 | 21.20 ±1.14 | 19.33 ±1.26 | 18.30 ±1.30 | 19.72 ±1.26 | 20.61 ±1.31 |
| Evolution of $^{14}CO_2$[e] after incubation with G-1-$^{14}$C | 1,097 ±69 | 1,119 ±73 | 1,231 ±69 | 1,112 ±70 | 1,049 ±58 | 796 ±49 |
| G-6-$^{14}$C | 641 ±35 | 635 ±41 | 681 ±44 | 654 ±48 | 652 ±48 | 540 ±47 |
| Ratio of $^{14}CO_2$ evolved G-1-$^{14}$C/G-6-$^{14}$C | 1.71 ±0.09 | 1.76 ±0.08 | 1.81 ±0.09 | 1.70 ±0.06 | 1.61 ±0.04 | 1.47 ±0.07 |

[a] Each value represents the mean ± standard error of 10 rats.

[b] Adrenalectomized rats were injected once every 24 hr for 5 days with either corn oil or the indicated dose of corticosterone and used 24 hr after the last injection.

[c] Amount of $CO_2$ evolved from bicarbonate buffer by acid end products during anaerobic glycolysis by cell-free preparation of testicular tissue ($\mu$l of $CO_2$/mg of protein/hr).

[d] Amount of $O_2$ consumed during aerobic catabolism of succinate by cell-free preparations of testicular tissue ($\mu$l of $O_2$/mg of protein/hr).

[e] Metabolism of glucose-1-$^{14}$C (G-1-$^{14}$C) and/or glucose-6-$^{14}$C (G-6-$^{14}$C) to $^{14}CO_2$ by teased testis tubules incubated at 32° for 2 hr in 3 ml of KRB buffer (pH 7.4) containing isotopic D-glucose (0.5 $\mu$Ci; 10 m$M$). Values expressed (dpm/mg of protein).

TABLE III. Effect of Adrenalectomy (ADX) and Corticosterone (CORT) on the Incorporation of Acetate-1-$^{14}$C into Testosterone-$^{14}$C by Rat Testicular Tissue *in Vitro*.

| Treatment[a] (mg) | No. of rats | Testosterone sp act[b] (cpm/$\mu$g of testosterone) |
|---|---|---|
| Control | 10 | 10,560 ± 450 |
| Sham ADX | 10 | 10,530 ± 509 |
| ADX | 10 | 10,902 ± 538 |
| + CORT, 0.75 | 7 | 10,697 ± 497 |
| 1.50 | 8 | 9,021 ± 519 |
| 3.0 | 7 | 7,797 ± 481 |

[a] Adrenalectomized were injected once every 24 hr for 5 days with either corn oil or the indicated dose of corticosterone and used 24 hr after the last injection.

[b] Each value represents the mean ± standard error. Testosterone-$^{14}$C synthetized by teased testis tubules incubated at 32° for 2 hr in KRB buffer (pH 7.4) containing sodium acetate-1-C$^{14}$ (25 $\mu$Ci; 2.5 m*M*) and D-glucose (10 m*M*).

tal testis carbohydrate averaged 1.80 mg/g in control rats and dropped to 1.39 mg/g after adrenalectomy ($p<0.05$). Corticosterone prevented the reduction in testicular carbohydrate following adrenalectomy as evidenced by fact that the carbohydrate content of the testis averaged 1.61 1.87, and 1.97 mg/g when adrenalectomized rats were treated with 0.75, 1.5, and 3.0 mg of corticosterone for 5 days. The average concentration of DNA, RNA, and the ratio of RNA:DNA in the testes of control, adrenalectomized, and corticosterone-treated rats was not significantly different ($p>0.25$).

After adrenalectomy, glycolytic activity of cell-free preparations of the testis was about 12% higher than that of control rats ($p<0.05$). By contrast, administration of 0.75 and 1.50 mg of corticosterone not only prevented the increase in glycolytic activity noted after adrenalectomy but maintained anaerobic glycolysis at about the same level as that found in the testes of control rats. However, 3 mg of corticosterone significantly reduced ($p<0.05$) testis glycolytic activity by approximately 13% below that found in control testes.

Neither adrenalectomy nor corticosterone therapy influenced the oxidation of succinate by cell-free preparations of testis suggesting that these treatments did not alter Krebs cycle activity. Testis tubules from adrenalectomized rats showed a tendency to produce more $^{14}CO_2$ from glucose-1-$^{14}$C and glucose-6-$^{14}$C than those of control rats, but these differences were not significant ($p \cong 0.08$). However, teased testis tubules from rats treated with 3 mg of corticosterone metabolized significantly less ($p<0.05$) glucose-1-$^{14}$C and glucose-6-$^{14}$C to $^{14}CO_2$ than those of control rats.

The ratio of $^{14}CO_2$ derived from the oxidation of glucose-1-$^{14}$C to that derived from glucose-6-$^{14}$C averaged 1.71 for testes from control rats, increased to 1.81 after adrenalectomy, and dropped significantly ($p<0.05$) to 1.47 in rats treated with 3.0 mg of corticosterone. The tendency for this ratio to approach unity suggests that the amount of glucose utilized via the hexose monophosphate shunt may have been reduced by treating adrenalectomized rats with corticosterone.

The amount of acetate-1-$^{14}$C incorporated into testosterone by testes of adrenalectomized rats was similar ($p>0.25$) to that observed in control rats, and rats treated with 0.75 and 1.5 mg of corticosterone. However, testes from rats treated with 3 mg of corticosterone synthesized significantly less ($p<0.05$) testosterone than control testes suggesting that corticosterone (3 mg) depressed testosterone biosynthesis (Table III).

*Discussion.* The present results demonstrate that treatment of adrenalectomized rats with 3 mg of corticosterone for 5 days reduces the capacity of the testis to catabolize glucose *in vitro* and to incorporate acetate-1-$^{14}$C into testosterone. These results coincide with morphological observations which indicate that the spermatogenic and steroidogenic functions of the testis may be lowered by hypercorticosteronism induced by chronic administration of ACTH (1) or by exposing adult rodents to high population densities (3). Changes in testicular metabolic activity were not accompanied by any significant alteration in either testis weight or the level of DNA and RNA. Similarly others noted that the weight and DNA and RNA

content of the testis remained unchanged despite marked alterations in testicular enzyme activity 48 hr after experimental cryptorchidism in rats (15). However, testes of rats treated with 3 mg of corticosterone for 5 days contained significantly less protein than that found in control testes. This reduction in testicular protein may be an early indication of the deleterious effects of corticosterone on the testis since others observed a significant reduction in testis protein 48 hr after rat testes were exposed to abdominal temperatures (15) or during the nonbreeding season in cotton rats (*Sigmadon hispidus*) (16). The effects of adrenalectomy on the testis consisted of a reduction in total carbohydrate concentration and an increase in the rate of anaerobic glucose catabolism. By contrast, treatment of adrenalectomized rats with corticosterone prevented the reduction in testis carbohydrate following adrenalectomy and depressed the capacity of cell-free preparations of the testis to catabolize glucose anaerobically. Moreover, these effects were achieved without any significant alteration in aerobic glycolysis. Taken together, the effects of adrenalectomy and corticosterone on testicular glucose metabolism, suggest that corticosterone modifies the capacity of the testis to utilize glucose primarily via the Embden-Meyerhoff pathway. These conclusions are consistent with the finding that cortisol influences glucose utilization by mouse epidermis *in vitro* by depressing anaerobic glycolysis rather than Krebs cycle activity (17).

Other assessments of the effects of adrenalectomy and corticosterone replacement on testicular glucose metabolism indicate that adrenalectomy tended to increase ($p \cong 0.08$) the amount of $^{14}CO_2$ derived from the decarboxylation of glucose-1-$^{14}$C and glucose-6-$^{14}$C. Corticosterone replacement (3 mg), on the other hand, significantly depressed ($p<0.05$) the amount of $^{14}CO_2$ produced from the catabolism of glucose-1-$^{14}$C and glucose-6-$^{14}$C but the reduction in the yield of $^{14}CO_2$ was primarily due to the capacity of corticosterone to inhibit the metabolism of glucose-1-$^{14}$C rather than glucose-6-$^{14}$C. Thus, these results suggest that corticosterone may have interfered with the decarboxylation of glucose in either the initial steps of the pentose phosphate pathway or in pyruvate decarboxylation or both. The significance of a reduction in the capacity of the testis to utilize glucose via the pentose phosphate pathway resides in the fact that it could impair the generation of NADPH and thus interfere with steroidogenic, lipogenic, and other reductive biosynthetic mechanisms. Evidence supporting this possibility stems from the observed reduction in the *in vitro* incorporation of acetate-1-$^{14}$C into testosterone following corticosterone (3 mg) replacement. The present suggestion that corticosterone may interfere with testosterone biosynthesis coincides with the observation that hypercorticosteronism induced by injecting rats with ACTH results in a reduction in the number of Leydig cells and involution of the accessory sex glands (1).

Whether the *in vitro* metabolic changes attributed to adrenalectomy and/or corticosterone replacement were due to a direct effect of corticosterone on the testis or innocently related to the overall metabolic effects of either adrenalectomy or corticosteroid therapy remains to be elucidated. Nevertheless, the results support the view that exogenous corticosterone may affect testis function by altering the capacity of the tissue to metabolize glucose and synthetize testosterone. The present findings also complement the observation of others who demonstrated that gonadal hormones may modify adrenocortical function (18).

*Summary.* Testicular metabolism was examined after adult rats were adrenalectomized and/or treated with corticosterone (0.75, 1.5, and 3.0 mg/day) for 5 days. The weight and DNA and RNA concentrations of testes were not affected by either adrenalectomy or corticosterone replacement but corticosterone (3.0 mg) reduced ($p<0.05$) testis protein and prevented the loss of testis carbohydrate noted after adrenalectomy. Anaerobic glucose catabolism (cell-free preparations) increased 12% ($p<0.05$) after adrenalectomy and decreased 13% ($p<0.05$) after corticosterone (3.0 mg) therapy but neither of these treatments affected aerobic glucose catabolism. Corticosterone (3.0 mg) depressed ($p<0.05$) the oxidation of glucose-

1-$^{14}$C and glucose-6-$^{14}$C to $^{14}CO_2$; whereas adrenalectomy showed a tendency to increase the activity of $^{14}CO_2$. Testosterone synthesis dropped ($p<0.05$) after corticosterone (3.0 mg) therapy but was not altered by adrenalectomy. The results suggest that adrenalectomy modifies testicular glucose catabolism and that corticosterone may affect the testis by reducing glucose utilization and testosterone synthesis.

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