Testosterone Regulation of 17β-Hydroxysteroid Dehydrogenases.* (25965)

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Guinea pig liver and kidney utilize diphosphopyridine nucleotide (DPN) or triphosphopyridine nucleotide (TPN) as the hydrogen acceptor in conversion of testosterone to 4 - androstene - 4,17 - dione(1,2). The coenzymes are linked with specific enzymes which function independently even in an homogenate(3). This report indicates that the activity of these enzymes in the kidney but not the liver is regulated by testosterone.

Methods and materials. DPN (Pabst) was used as purchased. Testosterone† for enzyme assay was further purified(4) and dissolved in water at 32 μg/ml(3). Testosterone propionate‡ was injected subcutaneously daily. Normal and control guinea pigs were injected with sesame oil. *Animals.* Guinea pigs (Zeimet Bio-Farms) were kept in screen bottom metal cages in an air conditioned room at 22-23°. They were fed Jim Dandy rabbit pellets with a supplement of 50 mg of ascorbic acid twice per week (Table I) or Rockland guinea pig diet with supplements of 30 g carrots and 25 mg ascorbic acid twice per week (Table II). Castration was performed under ether anesthesia. Guinea pigs were stunned by blow at base of skull and bled by severing blood vessels at neck. Liver and kidneys were removed and homogenized in ice-cold 0.25 M sucrose. Liver homogenate was diluted to 5 g/100 ml and the kidney to 10 g/100 ml. Dehydrogenases were determined as previously described(3). The reaction mixture contained: 1.0 ml 0.18 M sodium pyrophosphate pH 9.5, 1.8 ml testosterone 32 μg/ml and 0.2 ml of DPN (15 mg/ml) or TPN (5 mg/ml). Water was substituted for testosterone in the blank cell. The reactants were warmed to 40° prior to use. The reaction was initiated by addition of the ice-cold homogenate in the following amounts: DPN enzyme 0.06 ml liver, 0.15 ml kidney and TPN enzyme 0.08 ml liver, 0.05 ml kidney. The appearance of reduced coenzyme was recorded at 340 mμ in a Cary Model 14 Spectrophotometer at 38°. A unit of enzyme has been designated as a change in optical density of 0.001 per minute.

Results. Body and organ weights. Changes in body and organ weights after castration and testosterone propionate treatment were as expected(5,6,7). The androgen produced a small increase in body weight and restored the weight of seminal vesicles and prostates (Tables I, II). Liver and kidney weights were not significantly changed by either castration or testosterone propionate injections.

17β-hydroxysteroid dehydrogenases. Activity of enzymes of the liver was not significantly affected by castration or androgen treatment (Table I). Enzymes of the kidney, on the other hand, were decreased by castration and restored to normal by injections of androgens for 14 days. Similar results were obtained when doses of 5, 10 and 25 mg/day of testosterone propionate were administered for 65 days (Table II). Restoration of activity of the dehydrogenases preceded restoration of weight of the accessory sex organs (Tables I, II).

Discussion. The difference in response to castration and androgen treatment between the 17β-hydroxysteroid dehydrogenases of the kidney and liver suggest that these enzymes are associated with different functions in the 2 tissues. Many enzymes which apparently catalyse identical reactions have been demonstrated to respond differently in liver and kidney after castration or androgen administration(8). Furthermore, enzymes of the kidney have proved to be more responsive to concentration of androgen in the body(8,9). It is of interest, therefore, that the 17β-hydroxysteroid dehydrogenases of the liver were not affected while those of the kidney responded to castration and androgen administration.

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† Testosterone provided by Syntex.

‡ Testosterone propionate provided as Perandren by Ciba Pharmaceutical Products.

TABLE I. Effect of Castration and Testosterone Propionate on DPN and TPN 17β-Hydroxysteroid Dehydrogenases of Liver and Kidney of Male Guinea Pig.*

		17β-Hydroxysteroid dehydrogenases			
		Liver		Kidney	
	Sem. ves. & pros., g	DPN	TPN	DPN	TPN
		u/mg			
Normal	3.92	10.5	5.5	.94 ± .083	4.0 ± .27
Castrated†	.57	10.5	6.2	.65 ± .035	2.5 ± .14
" † + T P‡	2.54	10.3	5.9	.86 ± .063	4.0 ± .19

* Seven animals/group.

† Guinea pigs castrated at 58 days of age, 406-553 g body wt. Injections begun 36 days later and continued 13 to 16 days.

‡ Testosterone propionate (Perandren, 50 mg/ml) was inj. subcut. at 25 mg/day.

It should also be noted that administration of the androgen restored activities of the 2 enzymes to normal but not above normal level. Responses of the dehydrogenases are similar to those observed for arginase and alkaline phosphatase(9).

The presence and relative activity of the steroid dehydrogenases have been suggested as regulator mechanisms for the manifold effects of androgens and as an explanation for their differential effect on various tissues and among animal species(1,8). The guinea pig apparently uses 17β-hydroxysteroid dehydrogenases of the kidney and not the liver as a regulator to maintain a homeostatic mixture of androgens in its body. The doses of androgen were excessive and prolonged, yet accessory sex organs and muscles(7) were not stimulated to greater than normal levels. Localization of this mechanism in the kidney might be peculiar to the guinea pig. Administration of androgens alters activity of the 3β and 3α-hydroxysteroid dehydrogenases in the liver of the rat and other species but not that of the guinea pig(10). The adrenal cortical steroids also influence dehydrogenases of rat-liver. Administration of prednisone acetate or prednisolone acetate increased the activity of an adrenal cortical steroid dehydrogenase (change in Porter Silber reaction)(11). This increase in enzyme activity has been suggested as the mechanism for increased tolerance of glucocorticoids by patients after prolonged treatment with these steroids.

TABLE II. Effect of Dose and Extension of Testosterone Propionate Injections on 17β-Hydroxysteroid Dehydrogenase Activities of Guinea Pig Liver and Kidney.*

		17β-Hydroxysteroid dehydrogenases			
		Liver		Kidney	
	Sem. ves.† & pros., g	DPN	TPN	DPN	TPN
		u/mg			
Normal	6.49	9.5	5.1	1.09	3.5
Castrated	1.05	8.6	5.5	.77	2.3
Castrated‡ + 5 mg/day TP§	5.53	8.5‖	5.5‖	1.14	3.6
Castrated‡ + 10 mg/day TP§	6.04	10.5¶	5.1¶	1.07	3.9
Castrated‡ + 25 mg/day TP§	7.16	9.2	5.4	1.09	4.0

* Four animals/group.

† Kidney and liver wt not significantly changed.

‡ Castration performed at 68 days of age 549-698 g body wt.

§ Inj. begun day of castration and continued daily except Sunday for 65 days. Daily dose was contained in 0.5 ml of sesame oil.

‖ Single determination.

¶ Two determinations.

Summary. Castration of male guinea pigs produced a decrease in activities of DPN and TPN 17β-hydroxysteroid dehydrogenases of the kidneys. Subcutaneous injection of testosterone propionate for 14 days restored activities of kidney enzymes. Extension of injections to 65 days gave no further changes. Dehydrogenases of liver were not affected by castration or androgen administration. Ratio of DPN to TPN dehydrogenase was approximately 2:1 in liver and 1:4 in kidney. DPN enzyme activity of liver was approximately 10 times that of kidney.

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Erythrocyte Repopulation in X-Irradiated Recipients of Nucleated, Peripheral Blood Cells of Normal Mice. (25966)

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Unitarian and polyphyletic theories of blood cell formation have been discussed for many years(1). Many hematologists agree that the fixed stem cell may be pluripotent, but the capacity of more mature forms, and particularly of circulating cells, to dedifferentiate toward less-mature blast forms is not universally accepted. Studies with tissue culture technics have indicated that numerous morphological types can be derived from apparently pure blood cell cultures(2). Much information about blood cell differentiation may also be obtained by use of an *in vivo* culture system in mice, in which normal hematopoiesis has been destroyed by X radiation. In such mice, Merwin(3) showed that leukocytes of leukemoid blood produce immunologically competent cells, and Smith *et al.*(4) reported that leukemoid leukocytes differentiated into erythroblasts. Since leukemoid blood contains many immature cells, preliminary observations that circulating leukocytes from normal mice may produce erythroblasts in irradiated recipients are herein reported because of their important implications about the dynamics of normal hematopoiesis.

Materials and methods. A program was initiated to produce coisogenic strains of mice that differ at the hemoglobin locus. The scheme was essentially similar to that of Snell (5). The "single" type hemoglobin of strain C57BL mice was genetically introduced in strain BALB/c, RF, AKR, and 101 mice, which possess "diffuse" type hemoglobins, and the "diffuse" hemoglobin of 101 mice was introduced into C57BL mice. Technics of starch gel electrophoresis(6) and hemoglobin solubility(7) were used to identify mice that carried the introduced, genetically controlled, type of hemoglobin. At present, the coisogenic status of these strains varies from 5 to 8 generations of backcrossing.

Sixteen-week-old BC_5 coisogenic 101 mice, which were progeny of the BC_4 backcross to normal 101 mice and were heterozygous for diffuse and single hemoglobins, were given 600 r of X rays and used as recipients. X ray conditions were 250 kvp, 31.5 ma, hvl 0.43 mm of Cu; the dose rate in air was approximately 80 r/min, tissue to source distance was 93.7 cm. Donor mice were anesthetized with Nembutal, ventral skin was removed, and the sternum was excised to expose the heart. Cardiac blood was withdrawn by means of a hypodermic needle and syringe that contained 2% sodium citrate as anticoagulant. Twelve to 15 ml of blood was pooled from 10 normal 101 donors, 3-4 months old. White blood cells were separated in 15-ml centrifuge tubes by centrifugation at 600 X *g* for 15 minutes. The buffy coat and many underlying red cells were removed with a pipette and placed in Wintrobe hematocrit tubes to be centrifuged a second time at 600 X *g* for 20 minutes. Ap-

* Operated by Union Carbide Corp. for U. S. Atomic Energy Commission.