investigators demonstrated that in submitochondrial particles the succinate-dependent reduction of NAD^+ involves the 4B hydrogen of NADH, whereas during transhydrogenation, $NADP^+$ is reduced by the 4A hydrogen of NADH.

It is concluded that the energy-linked reduction of NAD^+ with succinate and the energy-linked transhydrogenase reaction of *R. rubrum* chromatophores do not share the soluble transhydrogenase factor as a common component.

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Cytochrome P-450 in adrenal mitochondria of male and female rats

Sex differences in adrenocortical function are well established. The adrenal is larger in the female, while adrenal venous corticosterone output is approximately twice as high in the female rat as in the male[1]. Liver homogenates from female rats reduce steroid ring A 3–10 times faster than those from males[2]. *In vitro* hydroxylation of 11-deoxycorticosterone to 18-hydroxy-11-deoxycorticosterone and corticosterone is stimulated by acute administration of estradiol *in vivo*[3], and this effect is apparently antagonized by testosterone *in vivo*[4].

Cytochrome P-450 acts as the terminal oxidase in many NADPH-dependent hydroxylation reactions involving steroids and drugs[5–7] including the 18- and 11β-hydroxylations of 11-deoxycorticosterone[8], which take place in adrenal cortex mitochondria[9]. The present study was undertaken to see what relationship exists between

the effects of estrogens and androgens on steroid hydroxylation previously noted and cytochrome P-450 levels in adrenal cortex mitochondria.

Adult Holtzmann rats weighing 150–200 g were anesthetized with ether before their adrenals were removed, chilled, and dissected free of fat. The glands were pooled, weighed, suspended in 1.15 % KCl and homogenized 3 or 4 at a time with 5 passes in a small Ten Broeck homogenizer. The homogenate was centrifuged at 900 × *g* for 10 min. The supernatant was spun at 12000 × *g* for 10 min, and the resulting mitochondrial pellet resuspended in 1.15 % KCl and resedimented. The mitochondria were suspended in 0.1 M phosphate buffer (pH 7.0) to give a final protein concentration of 0.5 mg/ml. Protein was determined by the method of LOWRY *et al.*[10].

Cytochrome P-450 level was measured using an Aminco-Chance spectrophotometer in the split beam mode or a Cary spectrophotometer Model 15. Two cuvettes contained 3 ml each of the mitochondrial suspension, which had previously been reduced with a few crystals of sodium dithionite, and steroid when appropriate. The baseline was balanced and recorded, and then CO was bubbled through the sample cuvette for 30 sec. A typical absorption peak at 450 nm resulted. Content of cytochrome P-450 was determined by measuring the $\Delta A_{450-480\ nm}$ and using a molar extinction coefficient of 91 $mM^{-1} \cdot cm^{-1}$ (ref. 11). Results were expressed as nmoles cytochrome P-450 per mg mitochondrial protein.

Orchiectomies and oophorectomies were performed 7–14 days prior to sacrifice. Long-acting testosterone (10 mg; Depo-Testosterone Cypionate, The Upjohn Co.) or estradiol (1 mg; Depo-Estradiol Cypionate, The Upjohn Co.) were injected subcutaneously at the time of surgery or 7–14 days prior to sacrifice in intact rats. Statistical analysis was by means of the Student's *t* test.

There was a significant difference in cytochrome P-450 levels in male and female adrenal mitochondria (Table I). Gonadectomy brought the groups to almost identical levels. Estradiol replacement in oophorectomized females did not raise the cytochrome P-450 levels significantly. However, 10 mg of depot testosterone administered to orchiectomized males caused distinct lowering of the level. Moreover, testosterone was also able to lower the cytochrome P-450 level in intact female rats but not in intact males.

Since testosterone is known to bind with cytochrome P-450[12,13], the question arose as to whether the lowering of the hemoprotein level was due to an actual decrease in the amount present or to an apparent decrease secondary to occupation of the binding sites by testosterone. Cytochrome P-450 levels were measured from a pool of adrenal mitochondria, with and without testosterone added *in vitro*. 50 μM testosterone in sample and reference cuvettes significantly lowered the apparent cytochrome P-450 level (Table II). 11-Deoxycorticosterone is another steroid known to bind cytochrome P-450[14,15], and it, at 120 μM, caused adrenal mitochondria containing 1.269 $\pm$ 0.409 nmoles cytochrome P-450 per mg protein to appear to contain 1.068 $\pm$ 0.290. Estradiol showed the same effect at both 12 and 120 μM (Table II).

BROWNIE *et al.*[8], studying methylandrostenediol-induced hypertension, and SKELTON *et al.*[16], examining rats treated chronically with testosterone, found decreased adrenal mitochondrial cytochrome P-450 at the same time that 11β- and 18-hydroxylating capacity was diminished. This agrees with the present results in that the intact male, which has constantly circulating levels of testosterone which are 5–50 times higher than the female, has a lower cytochrome P-450 level. Castration brought

TABLE I

CYTOCHROME P-450 LEVELS IN RAT ADRENAL MITOCHONDRIA

Animals were castrated 7–14 days prior to sacrifice. Estradiol and testosterone, in the doses indicated, were administered as a subcutaneous depot 7–14 days before sacrifice. Each experiment involved 3–6 rats per group.

Group	*Condition*	*Treatment*	*Number of experiments*	*nmoles P-450 per mg protein (mean ± S.D.)*	*P*
Male	Intact	—	9	1.410 ± 0.554	<0.05
Female	Intact	—	6	2.098 ± 0.541	
Male	Orchiectomized	—	7	1.604 ± 0.402	<0.025
Male	Orchiectomized	10 mg testosterone	7	1.060 ± 0.317	
Female	Oophorectomized	—	6	1.640 ± 0.476	>0.2
Female	Oophorectomized	1 mg estradiol	6	2.007 ± 0.712	
Male	Intact	—	9	1.410 ± 0.554	0.5
Male	Intact	1 mg estradiol	7	1.554 ± 0.371	
Female	Intact	—	6	2.098 ± 0.541	<0.05
Female	Intact	10 mg testosterone	6	1.507 ± 0.299	

TABLE II

RAT ADRENAL MITOCHONDRIAL CYTOCHROME P-450 LEVELS IN THE PRESENCE OF STEROIDS ADDED *in vitro*

In each experiment, mitochondria were obtained from 6 intact female rats and pooled. Control and test levels of the cytochrome P-450·CO complex were determined from the same mitochondrial pool, the only difference being that both sample and reference test cuvettes were balanced while containing steroid before addition of CO to the sample cuvette. *P* values were determined on the paired samples.

Steroid	*Concn. (μM)*	*Number of experiments*	*nmoles P-450 per mg protein* *Control ± S.D.*	*Test ± S.D.*	*P*
Testosterone	10	5	1.551 ± 0.316	1.427 ± 0.328	>0.1
	50	5	1.551 ± 0.316	1.254 ± 0.278	0.01
11-Deoxycorticosterone	12	8	1.269 ± 0.409	1.285 ± 0.586	>0.5
	120	8	1.269 ± 0.409	1.068 ± 0.290	<0.05
Estradiol	12	7	1.130 ± 0.270	1.053 ± 0.250	<0.025
	120	7	1.224 ± 0.264	1.060 ± 0.295	0.025

about enough of a rise in the male levels and a fall in the female levels to cause the adrenal mitochondrial cytochrome P-450 content to be identical in both sexes. Moreover, administration of testosterone to intact females or orchiectomized males induced a significant drop in adrenal cytochrome P-450, confirming the findings of BROWNIE *et al.*[8] and SKELTON *et al.*[16] and suggesting a direct androgen effect on the cytochrome.

BURROW[17] found that testosterone was a competitive inhibitor in the conversion of corticosterone to aldosterone. CO inhibits the 11β-hydroxylation of 11-deoxycorticosterone[9,18] and the hydroxylation of testosterone[19]. Evidence points to the

occupation of the same site by some inducers of Type I spectra[13,20] and in particular testosterone and 11-deoxycorticosterone, each of which interferes with the formation of a spectrum in the presence of the other[21]. One mechanism producing the apparent lower levels of cytochrome P-450 in male or testosterone-treated rats may therefore be combination of testosterone with the cytochrome to prevent formation of CO spectra. It may also prevent binding and hydroxylation of other steroids.

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