On the enzymic conversion of Δ^4-3-hydroxy steroids to Δ^4-3-ketones

Although the occurrence of Δ^4-androstene-3β,17β-diol or Δ^4-androstene-3α,17β-diol in natural sources has never been reported, these steroids are readily converted to testosterone by rat and chick liver homogenates[1]. The enzyme activity was first described as being principally in the 5,000 × *g* supernatant of rat liver homogenates and of rat and chick liver acetone powders. Further studies show that the activity is found in the soluble fraction following centrifugation at 78,000 × *g*. The activity is precipitated from the supernatant with ammonium sulfate between 0.4 and 0.55 saturation.

The pH optimum for the conversion of the Δ^4-3-hydroxyl to the Δ^4-3-ketone was between pH 7.5 and pH 8.3 using either a phosphate or tris(hydroxymethyl)aminomethane buffer. In the subsequent metabolism of the formed testosterone, greater amounts of Δ^4-androstene-3,17-dione and reduced ring-A saturated 17-ketosteroids were also formed at the higher pH ranges. Since the pH optimum for 17-ketosteroid formation was shown previously to be 8.3 by SWEAT *et al.*[2], the increased formation of these end products can be ascribed to the optimal pH for the formation of testosterone as well as for the 17-ketones.

Both DPN and TPN were equally effective in the conversion of the Δ^4-3-hydroxyl to the Δ^4-3-ketone for the more purified tissue preparations, although DPN was more effective for the 5,000 × *g* supernatant preparation. An absolute requirement for the pyridine nucleotides has not been demonstrated, since tissue incubated without added pyridine nucleotide still contains some activity.

Incubation of the 3α- and 3β-isomers of Δ^4-pregnenolone*[3] with the 78,000 × *g* supernatant fluid of rat liver resulted in the formation of progesterone. The incubation product was extracted with ethyl acetate and chromatographed on paper in the ligroin-propylene glycol system for 6 h. The major zone gave an orange color with dinitrophenyl hydrazine, a blue color with the Zimmermann reagent, and absorbed in the ultraviolet light at 240 mμ. The zone was chromatographed on silica gel, and crystals were eluted with benzene-ethyl acetate, 9:1. Recrystallization from aqueous methanol gave crystals, m.p. 124–8° C, which did not depress the melting point of authentic progesterone, m.p. 124–8° C. The identity of the substance as progesterone was verified by infrared analysis.

TABLE I

THE CONVERSION OF Δ^4-ANDROSTENE-3β,17β-DIOL TO TESTOSTERONE BY THE SUPERNATANT OF RAT LIVER ACETONE POWDER

Incubation volume of 5 ml consisted of 0.002 *M* Δ^4-androstene-3β,17β-diol, 0.003 *M* pyridine nucleotide, and 2 g equivalent tissue wet weight in 0.1 *M* phosphate buffer, pH 7.4. The flasks were incubated in air at 37° for 30 min.

Tissue preparation	*Pyridine nucleotide added*	*Percent conversion of Δ^4-diol*
5,000 × *g* super	+ DPN	42.3
	+ TPN	20.9
	—	8.6
78,000 × *g* super	+ DPN	45.6
	+ TPN	47.8

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