Preliminary Notes

Inhibition of 3α-hydroxysteroid-mediated transhydrogenase of rat-liver homogenate by Δ^4-3-oxosteroids

The 3α-hydroxysteroids with saturated ring A, *e.g.*, androsterone, and 17β-hydroxysteroids, *e.g.*, estradiol, have been shown to function *in vitro* as co-enzymes for hydrogen transfer in enzyme systems[1-3]. We have found an apparent inhibition of 3α-hydroxysteroid-mediated transhydrogenase from rat-liver homogenate by Δ^4-3-oxosteroids, *e.g.*, testosterone. The present report deals with the structural specificity of the inhibitory steroids.

A 3α-hydroxysteroid dehydrogenase was prepared as a 50–70 % ammonium sulfate fraction of adult-male-rat liver by the method of HURLOCK AND TALALAY[2]. Preparations obtained from adult-female-rat livers were also active. 3α-Hydroxysteroids and 3-oxosteroids with a saturated ring A mediated hydrogen transfer at rates comparable to those previously reported[2]. In this study androsterone ($4 \cdot 10^{-6}$ *M*) was the principal steroid used to mediate transhydrogenation. Testosterone was purified extensively by chromatography, recrystallization, and sublimation. The purified testosterone dissolved in dioxane was added to the reaction vessel either at the onset or 10 min after initiation of hydrogen transfer with androsterone (Fig. 1). Inhibition of transhydrogenation occurred in both instances. Testosterone also inhibited hydrogen transfer mediated by androstane-3,17-dione, 5β-androstane-17β-ol-3-one, 5β-androstane-3,17-dione, and 5α-pregnane-3α,20α-diol.

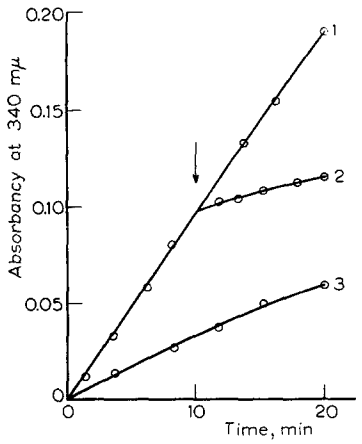

Fig. 1. Testosterone inhibition of androsterone-mediated transhydrogenase. The complete system contained in a volume of 3 ml: 200 μmoles tris(hydroxymethyl)aminomethane buffer, pH 7.5, 1 μmole DPN, 10 μmoles glucose 6-phosphate, 1 μmole $MgCl_2$, excess of glucose 6-phosphate dehydrogenase, 0.03 μmole TPN, 3 μmoles ethylenediaminetetraacetic acid and 0.1 ml (6.3 mg protein) of 50–70 % ammonium sulfate fraction of rat-liver homogenate. 3.5 μg androsterone in 0.01 ml dioxane were added to initiate the reaction. 3.3 μg testosterone in 0.01 ml dioxane were added at times 0 and 10 min to vessels 3 and 2, resp. Measurements were made of absorbancy at 340 mμ against a control cuvette containing all ingredients except steroid. Temp. 25°.

All steroids tested that had a 3-oxo group and conjugated double bond were effective inhibitors of the androsterone-mediated transhydrogenase. Greatest inhibition was obtained with $\Delta^{1,4}$-3-oxosteroids (1-dehydrotestosterone and 1-dehydrotestololactone), complete inhibition occurring at a concentration of $1 \cdot 10^{-6}$ *M*. The

Abbreviations: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

inhibitory concentration was less than that of androsterone ($4 \cdot 10^{-6} M$) used to mediate hydrogen transfer. An inhibitory influence was noted at a concentration as low as $3 \cdot 10^{-7} M$. In diminishing order the inhibitory potency of the Δ^4-steroids was: 3-oxo > 3α-hydroxy > 3β-hydroxy. Estradiol, 17α-ethyl-19-nortestosterone, hydrocortisone, and 11-dehydrocorticosterone were less effective inhibitors than 4-androstene-3,17-dione. The inhibitory influence produced by progesterone and 11-deoxycorticosterone was transitory with recurrence of hydrogen transfer after 10 min. The recurrence was probably due to saturation of Δ^4-bond by the Δ^4-3-ketosteroid hydrogenases in the system[4-7]. TOMKINS[8] reported that α,β-unsaturated steroids inhibited 3α-hydroxysteroid dehydrogenase of rat-liver homogenate.

The degree of inhibition of 3α-hydroxysteroid-mediated transhydrogenation correlated with the quantity of testosterone added. The extent of inhibition was greater when testosterone was added 10 min after mediation of the reaction than at the start. Testosterone inhibition of androsterone-mediated transhydrogenase depends on the relative concentration of DPN and TPN. Preliminary study indicates that a testosterone-dependent inhibitory substance may be present in the rat-liver homogenate.

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Cellulose polysulfatase, an enzyme attacking cellulose polysulfate and charonin sulfate

An enzyme hydrolysing sulfuric ester bonds in cellulose polysulfate and charonin sulfate has been found in the liver extract of a marine gastropod, *Charonia lampas* (*Tritonalia sauliae*). Since chondroitin sulfate and amylose polysulfate are scarcely hydrolysed by the enzyme preparation, we propose to name the new sulfatase "cellulose polysulfatase".