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Metabolism of Testosterone and Its Conjugates by Female Rat Liver*

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An interesting aspect in the study of steroid conjugates is the further metabolism of these compounds without prior hydrolysis. In the preceding papers, we compared the metabolic transformation of testosterone, testosterone 17-glucosiduronate (TGA), testosterone 17-N-acetylglucosaminide (TNAG) and testosterone 17-sulfate (TS) by incubation with cell-free preparations of male rat liver, and demonstrated metabolic differences among these compounds. Thus, TGA and TNAG were not good substrates for microsomal Δ^4-5α-hydrogenase as well as for hydroxylases, while they were better substrates for soluble Δ^4-5β-hydrogenase than testosterone. In contrast, TS was metabolized more readily by microsomal Δ^4-5α-hydrogenase than by soluble Δ^4-5β-hydrogenase, but not a good substrate for microsomal hydroxylases. Testosterone was extensively metabolized by the same enzymes.

On the basis of enzymic studies, the sex difference in Δ^4-5β-hydrogenase activities of rat liver was well proved by using free steroids as substrates. In both sexes, the Δ^4-5α-hydrogenase is located mostly in the microsomal fraction and the Δ^4-5β-hydrogenase is present in the soluble fraction. In female rat liver, the Δ^4-5β-hydrogenase activity is much lower than that of the Δ^4-5α-hydrogenase, thus 3-oxo-Δ^4-steroids being mostly metabolized to 5α-steroids. Quite recently, Schriefers, *et al.* observed sex-specific aglycone patterns by incubating testosterone with rat liver slices, followed by examination of the glucosiduronate fraction. Both 5α- and 5β-steroid glucosiduronates were produced in the male, while only 5α-steroid glucosiduronates were present in the female. In the present paper, the metabolism of testosterone, TGA and TS was studied by incubation with cell fractions from female rat liver, and their metabolic patterns were compared with those of male rat liver.

It is apparent from the present study that TGA and TS undergo substantial metabolic transformation as conjugate by the female rat liver preparations as does testosteorne.

The incubation of testosterone, TGA and TS with 105000xg supernatant fluid in air showed that they were good substrates for Δ^4-hydrogenases and 3α-hydroxysteroid oxidoreductase. Testesterone was metabolized to hydroxylated polar

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steroids (5%), 5β-androstane-3α, 17β-diol (15%) and 5α-androstane-3α, 17β-diol (28%), 42% of testosterone being recovered unchanged. TS gave the similar type of metabolic pattern to testosterone after solvolysis : hydroxylated polar steroids (4%), 5β-androstane-3α, 17β-diol (14%), 5α-androstane-3α, 17β-diol (41%). The increased formation of the 5α-steroid resulted in the reduced recovery of unchanged testosterone (16%), in comparison with the incubation of testosterone. In contrast, TGA produced 5β-androstane-3α, 17β-diol (78%) in high yield and hydroxylated polar steroids (7%) after hydrolysis with β-glucuronidase. Formation of 5α-steroid was not observed when testosterone and TS were incubated with the 105000xg supernatant fluid of male rat liver in the same condition. Presence of Δ^4-5α-hydrogenase as well as Δ^4-5β-hydrogenase in the 105000xg supernatant fluid of female rat liver was reported by Forchielli, *et al.*, using androst-4-ene-3, 17-dione as substrate. However, the predominant production of 5β-steroid in the incubation with TGA was quite consistent with the observation on male rat liver. These findings indicate that TGA is quite readily metabolized by the Δ^4-5β-hydrogenase. Enzymic mechanism for the stereospecific hydrogenation of the Δ^4-double bond of TGA is not clear. It could be explained analogously according to Gerhards, *et al.* that the bulky and polar sugar group attached at 17β-position of testosterone makes the α-side of the steroidal ring A approach preferentially to the surface of the 5β-enzyme, followed by the transfer of the hydride ion from coenzyme NADPH to the β-side of the Δ^4-double bond, though there is no definite evidence that the same 5β-enzyme can reduce both testosterone and TGA.

The incubation of testosterone, TGA and TS with microsomal fraction in air revealed that they were metabolized by Δ^4-5α-hydrogenase, 3α-hydroxysteroid oxidoreductase as well as hydroxylase. Testostrone was transformed in 88% yield to hydroxylated polar steroids, as was observed with male rat liver. TGA and TS were metabolized as conjugate and their metabolites were determined after hydrolysis. Thus, TS was converted to hydroxylated polar steroids (47%), 17β-hydroxy-5α-androstan-3-one (16%) and 5α-androstane-3α, 17β-diol (12%). Only 3% of testosterone was recovered unchaged. While, TGA gave hydroxylated polar steroids (7%) and 5α-androstane-3α, 17β-diol (17%), 57% of testosterone being recovered unchanged in this case. Furthermore, most of the metabolites of testosterone, TGA and TS were compared after incubation with microsomal fraction under carbon monoxide atmosphere whereby hydroxylase activities were suppressed. Testosterone produced hydroxylated polar steroids (16%), 5α-androstane-3α, 17β-diol (38%) and androsterone (27%). TS was transformed to hydroxylated polar steroids (22%), 5α-androstane-3α, 17β-diol (40%) and 17β-hydroxy-5α-androstan-3-one (13%), only a small amount (3%) of testosterone being recovered. On the other hand, TGA gave essentially a similar result to that of the incubation in air ; hy-

droxylated polar steroids (4%), 5α-androstane-3α, 17β-diol (11%) and testosterone (68%).

Production of a large quantity (47%) of hydroxylated polar steroids from TS in the incubation under air is in marked contrast to that obtained with male rat liver, whereby only 6% of TS was converted into hydroxylated polar steroids. Jacobson, *et al.* had reported that testosterone was more readily hydroxylated by the microsomal fraction of male rat liver than that of the female. Possible interpretation of these striking discrepancies observed between testosterone and TS in both sexes is not available at present. In view of the fact that some steroid sulfates can serve as an active intermediate in the metabolism, these results may be of any physiological significance of TS or related steroid sulfates in the female rat, but await further elucidation.

Formation of 17β-hydroxy-5α-androstan-3-one, a potent androgen, was demonstrated only when TS was incubated with the microsomal fraction of female rat liver. The catabolic sequence of 3-oxo-Δ^4-steroids is well established to proceed mainly *via* 4,5-dihydro-3-oxosteroids to saturated 3-hydroxysteroids by the consecutive action of Δ^4-hydrogenases and 3-oxosteroid oxidoreductases. Thus, in most cases, 3-oxo-5α-steroids were preferentially reduced to 3-hydroxy-5α-steroids in our incubation condition.

The sex difference in the Δ^4-5α-hydrogenase activity was clearly demonstrated by the fact that the 5α-enzyme of female rat liver produced 5α-steroid from TGA though in a small amount, whereas the 5α-enzyme from male rat liver did not yield any 5α-metabolite. Finally, metabolism of testosterone also indicated sex-specific patterns of microsomal 3-oxosteroid oxidoreductase activities, since testosterone was transformed to 3α-hydroxy-5α-steroid by the female, while it was converted to 3β-hydroxy-5α-steroid by the male. These results are in good agreement with the perfusion experiment of testosterone with the rat liver.