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Metabolism of Testosterone Sulfate in the Rat : Analysis of Biliary Metabolites*

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Metabolism of testosterone-^{14}C-17-sulfate in humans was first investigated by Baulieu and his coworkers, whereby no ring A saturated $C_{19}O_2$ steroids nor estrogens were isolated from the urinary conjugates, the injected sulfate being recovered unchanged from the urine in very low yield. Recently, a more detailed study on the metabolism of testosterone-^{3}H-17-sulfate-^{35}S in humans was reported by Knapstein and his coworkers. Only a few percent of the injected radioactivity was excreted in the urine. Analysis of the liver vein plasma revealed the production of 16-oxygenated steroid and 5α-$C_{19}O_2$ steroid sulfates with the same ^{3}H/^{35}S ratio as the injected dose, indicating the direct metabolism of testosterone sulfate. Thus, little is known about the *in vivo* metabolism of testosterone sulfate. *In vitro* studies with the rat liver preparation demonstrated that testosterone sulfate could be metabolized without hydrolysis of the ester linkage by Δ^4-5α- and Δ^4-5β-hydrogenases as well as 3α- and 3β-hydroxysteroid-oxido-reductases. These observations prompted us to investigate the *in vivo* metabolism of testosterone sulfate in the rat. Tracer amounts of testosterone-^{3}H-17-sulfate [^{3}H-TS] and testosterone-^{14}C [^{14}C-T] were injected together into male and female rats and the biliary metabolites were isolated and identified.

The results of the present investigation indicate that ^{3}H-TS can be extensively metabolized in the rat. The major portion of the metabolites was rapidly excreted in the bile, confirming that the bile is an important excretory route for testosterone metabolites in the rat. The metabolism of TS was remarkably affected by the sex and significant differences existed between TS and testosterone with respect to biotransformation and conjugation.

The metabolites of the injected ^{3}H-TS were excreted predominantly in the diconjugate fraction as disulfates in both sexes, whereas those of ^{14}C-T were distributed in various conjugate fractions. Solvolysis of the disulfates revealed the sex-specific aglycone pattern : The major portion of ^{3}H-TS was metabolized to 5α-androstane-3β, 17β-diol in the male, whereas the sulfate was converted into 5α-androstane-3α, 17β-diol and polar steroids in the female. High ^{3}H/^{14}C ratios of these 5α-steroids indicate that conversion of ^{14}C-T to these metabolites was minor in the female and almost none in the male. No 5β-metabolites were identified in

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both sexes. These *in vivo* findings of the preferential 5α-reduction of TS are in good agreement with the previous *in vitro* studies with the rat liver preparation. Incubation of ^{3}H-TS with male liver microsomes produced exclusively 5α-androstane-3β, 17β-diol 17-sulfate, whereas the female liver microsomes yielded the corresponding 3α-epimer and polar steroids. Wu and Mason demonstrated that TS was a better substrate for the female rat liver Δ^{4}-5α-hydrogenase than testosterone. From these studies, the following catabolic sequence of TS in the rat could be reasonably formulated. TS must undergo direct metabolism by liver microsomal Δ^{4}-5α-hydrogenase and 3α- or 3β-hydroxysteroid-oxido-reductase to 5α-androstane-3α, 17β-diol 17-sulfate or to the corresponding 3β-epimer, which should be consecutively conjugated with sulfuric acid to the 3,17-disulfate by the sulfokinase located in the soluble fraction of the liver cell. The resultant disulfate should be rapidly excreted in the bile. In sharp contrast, testosterone-17-glucosiduronate, another type of testosterone conjugate, yielded predominantly 5β-androstane-3α, 17β-diol 17-glucosiduronate in the rat. Jacobson and Kuntzman reported that testosterone was more readily hydroxylated by microsomal hydroxylases of the male rat liver than that of the female. However, the previous *in vitro* and present *in vivo* studies demonstrated that the production of a considerable quantity of polar steroids was a remarkable feature in the female rat, though the nature of these metabolites was not elucidated. Knapstein and his coworkers presented evidence that TS could be directly metabolized to 16-oxygenated steroids in humans. Very recently, Gustafsson and Sundberg demonstrated that 5α-androstane-3α, 17β-diol 3, 17-disulfate was readily hydroxylated only by female rat liver microsomes to the corresponding 15β-hydroxysteroid. In view of the fact TS was metabolized to 5α-androstane-3α, 17β-diol 3, 17-disulfate in the female rat, the elucidation of the polar steroids seems to be of considerable interest and is going to be investigated in near future.

Androsterone was excreted as monoglucosiduronate and monosulfate in the female rat bile and the low ^{3}H/^{14}C ratios indicated that it was mainly derived from testosterone as reported previously.

The marked difference in the biotransformation and conjugation between testosterone and its conjugate implies that the catabolic pathway of testosterone involving the initial conjugation with sulfuric acid might be a minor route in the female and almost absent in the male.

The steroid metabolism is remarkably affected by genetic, physiological, and environmental factors, resulting in changes in the pattern of metabolism. Recently, Ermini and his coworkers presented evidence that the metabolism of ^{14}C-TS in the human foetal liver could be characterized by the formation of 5β-steroid 17-sulfates. These authors also demonstrated that simultaneously administered ^{3}H-T

produced predominantly 5β-steroid 3-sulfates. These findings differ from the preferential 5α-reduction of TS in the rat. In the previous papers, we demonstrated that the rat liver soluble Δ^4-5β-hydrogenase could metabolize TS to some extent to 5β-steroids, though TS was readily metabolized by the liver microsomal Δ^4-5α-hydrogenase. Thus, these discrepancies might be ascribable to the weak activity of the Δ^4-5α-hydrogenase and/or to the presence of active Δ^4-5β-hydrogenase in the foetal liver.