THE METABOLISM OF STEROIDS BY HUMAN MAMMARY TISSUES

Ronald A. F. Couch

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SUMMARY

1. Human mammary tissue was incubated *in vitro* with [7-^{3}H] dehydroepiandrosterone sulphate (DHA-sulphate) and in agreement with other investigators this steroid conjugate was metabolized to DHA and other steroid products. Sulphatase activity was greater in the malignant than the non-malignant tissues and was found to be a function of the tissue cellularity. One of the major products, a "polar steroid" necessitated identification.

2. The "polar steroid" was identified principally as 7α-hydroxy DHA by chemical modification techniques and co-crystallization of the purified steroid metabolite with carrier 7α-hydroxy DHA. This carrier required synthesis and characterization.

3. Similarly incubation experiments carried out with the substrates [7-^{3}H] DHA and [7-^{3}H, ^{14}C] DHA showed that the tritium label was partially displaced from the C_7 position as a result of 7-oxygenation. The metabolites 7-keto DHA and 7β-hydroxy DHA were identified. When the substrate was [1,2-^{3}H] DHA the label remained on the steroid and 7-keto DHA as well as 7α-hydroxy DHA were identified as major products.

4. During the incubations with [7-^{3}H] DHA and [1,2-^{3}H] DHA the C_{19} steroids androstenediol, androstenedione, androstanedione and testosterone were isolated as metabolites. Evidence was obtained for the minimal metabolism of DHA to DHA-sulphate and to estradiol. No metabolic pathways were found to occur selectively in the malignant or non-malignant tissues.

5. Other Δ^5-3β-hydroxy steroids (cholesterol sulphate and pregnenolone sulphate) were also found to be suitable substrates for the sulphatase and 7α-hydroxylase enzyme systems. The 7α-hydroxy and 7-keto metabolites of both substrates were identified. However, the Δ^4-3-ketosteroid androstenedione and the 5α-reduced steroid dihydrotestosterone did not undergo 7-oxygenation, but were metabolized to other steroids containing two oxygen functions.

6. High specific activity [1,2-^{3}H] 7α-hydroxy DHA and [1,2-^{3}H] 7β-hydroxy DHA were synthesized and incubated *in vitro* with human mammary tissues. These substrates were metabolized principally to 7-keto DHA and minor quantities of the respective 3β,7,17β-triols. 7α-Hydroxy DHA was sulpho-conjugated at the 3β position. Also there was evidence for an epimerase converting 7α-hydroxy DHA to 7β-hydroxy DHA and vice versa. There was no evidence for the metabolism to 7-dehydro DHA, the B-ring unsaturated estrogens or 7-hydroxylated estrogens, and no evidence for a selective pathway of metabolism in malignant or non-malignant tissues.

7. It was concluded that 7-oxygenated derivatives of DHA were not precursors of potent physiologically active steroids. Alternatively, this metabolic pathway may act either as an alternative to the production of androgens and estrogens from DHA or, by the production of 7α-hydroxyandrostenediol, may act to inhibit the antagonistic effect of androstenediol towards estradiol at the estrogen receptor.

8. The sulphatase activity previously found to hydrolyse the sulphate conjugates of C_{19}, C_{21} and C_{27} steroids was also found to cleave estrone sulphate. The principal metabolites were estrone and estradiol.

9. The apparent Michaelis Constants were determined for the hydrolysis of estrone sulphate, DHA-sulphate, androstenediol sulphate and pregnenolone sulphate by human mammary tissue sulphatase(s). Apparent Michaelis Constants were also obtained for the 7α-hydroxylation of DHA and androstenediol and for the conversion of 7α-hydroxy DHA to 7-keto DHA. This data indicates that all these metabolic steps can occur at the physiological concentration of the steroid substrate.

10. The sulphatase and the 7α-hydroxylase enzyme systems were found to be located in the microsomal fraction of the human mammary tissue cellular preparation.

11. Plasma steroids directly influence the steroid micro-environment of body tissues. However, this study has shown that in mammary neoplastic lesions this environment is further determined by enzymic steroid reactions carried out by the neoplastic tissue. These pathways of steroid metabolism involve the production of estrogenic and androgenic steroids, estrogen agonist and antagonist steroids, steroid sulphate conjugates and steroids which are probably non-hormonal.

ABBREVIATIONS

n	as in '[7(n)-^{3}H]' denotes that the steroid was nominally labelled with tritium at the C_7 carbon atom.
B	as in 'B-ring' denotes the steroid 'hexagon' formed by the C_5 to C_{10} carbon atoms.
$CDCl_3$	deuterated chloroform
C_5D_5N	deuterated pyridine
MeOH	methanol
EtOH	ethanol
$NaBH_4$	sodium borohydride
TCA	trichloroacetic acid
$\underline{M}^{+\cdot}$	mass of the molecular ion
m/z	mass of molecular ion fragments
ε	extinction coefficient
d.p.m.	disintegrations per minute
f	femto
λ	ultraviolet absorption maxima
ν	infrared absorption maxima
m.p.	melting point