E > 90% within 3 days, presumably by reducing endogenous D/DS. E (mainly estrone) was determined by RIA after enzyme hydrolysis of conjugates. Increments in E, estimated from the difference in E during 5 days pre- and post-treatment, were [μg mean (range), number of expt.]: Control A (no treatment), −47 (−462/+472), 8; Control B (5 mg estradiol−17β), +2465 (+1358/+3537), 7; 100 mg D, +3206 (+1610/+5321), 7; 100 mg D + betamethazone, +2244 (+1655/+2824), 2; 100 mg DS (≡74 mg D), +35 (−1587/+659), 7; 100 mg DS + betamethazone, +107 (+46/+169), 2. Thus, preferential use of tracer D is unrelated to pool size, and variations in D availability and placental sulfatase activity may both contribute to regulation of estrogen production.

84. The biochemical basis of placental sulfatase deficiency

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The human placenta is normally a rich source of the enzymes 3β-steroid sulfatase and arylsulfatase but pregnancies have been recognized recently with deficiency in the activity of these enzymes. Fetal growth and development are normal but estrogen levels are extremely low since the placenta is unable to metabolise the estrogen precursors androst-5-en-17-one-3β-yl sulfate (DHEAS) and 16α-hydroxyandrost-5-en-17-one-3β-yl sulfate. In attempting to establish the biochemical mechanism of the enzyme defect we have investigated the possibility that lack of enzyme activity was due to the presence of a sulfatase inhibitor. Sulfatase activity was determined kinetically from the hydrolysis of DHEAS-7α^3H by microsomal preparations under optimum conditions. Microsomal preparations from affected placentas exhibited sulfatase activity. Ultrasonic (100 W, 24 k Hz) treatment of the microsomes directed at disrupting a possible enzyme-inhibitor complex failed to increase the sulfatase activity of the preparation. Addition of 105,000 μg supernatant from affected placental tissue to the incubate did not inhibit sulfatase activity of normal placental microsomes. It is concluded that placental sulfatase deficiency is likely to be due to deficiency of enzyme rather than the presence of a sulfatase inhibitor.

85. Human placental Δ_5-3β-hydroxysteroid dehydrogenase: intracellular distribution, substrate specificity and inhibition

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Δ_5-3β-hydroxysteroid dehydrogenase (Δ_5-3β HSD) activity has been assayed either by spectrophotometric method or by use of radioactive substrates. The enzymatic activity is equally distributed between mitochondrial and microsomal fractions verified by electronic microscopy. The specific activity is similar in both fractions, as well as the K_m for NAD (5×10^{-5}M), for the substrate, and the optimal pH. But the enzymatic activity is different with C_{21} or C_{19} natural or synthetic substrates; the optimal pH is 7·4–8 for C_{21} steroids and 10–10·2 for C_{19} steroids. The Δ_5-3β HSD is strongly inhibited by Δ_4-3-oxosteroids (progesterone and 4-androstenedione) and the inhibition seems to be of the competitive type. Oestrogens and cyclic AMP have also an inhibitory action. These results suggest that human placental Δ_5-3β HSD which has identical characteristics in mitochondrial and microsomal fractions has different properties with C_{21} or C_{19} steroid substrates, and that endogenous steroids play a physiological role in the regulation of the enzymatic activity.

3F. Steroid biosynthesis: Non endocrine tissues

86. Testosterone biosynthesis by human skin

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Steroid metabolism in the human skin was studied using purified progesterone-4-^{14}C as a testosterone and 17α-hydroxyprogesterone precursor. Skin minces were placed in Eagle's medium, with penicillin and streptomycin, and thereafter incubated for a five days period at 37 C. Neither PPLO or other bacteria were detected as contributing factors to steroid metabolism. Organic extracts were paper chromatographed and labelled steroid detected by a gas-flow strip scanner. Separation, elution and radioactivity quantification was performed until constant specific activities of the compounds were obtained. 17α-Hydroxy-4-pregnene-3,2 - dione and 17α-hydroxy-4-androsten-3-one yields accounted for less than 1% of the incubated precursor. The remaining metabolites have been previously described. Skin steroid metabolic pathways are becoming established. 17α-Hydroxy-4-pregnene-3, 20-dione might constitute an intermediate step to testosterone; both may be formed either as a compensatory route or just as a tissue response to form adaptive enzymes.

87. *In vitro* synthesis of active sex steroids in normal and inflamed human gingiva

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This study employed 4 groups (each of 12 samples) of normal and inflamed human gingival tissue of male and female subjects. 300 mg of tissue slices were incubated, separately, in 3 ml of 0·1 M potassium phosphate buffer (pH 7·4) with 50 μCi of androstenedione-1,2-^{3}H (Δ^4A) or estrone-2,4,6,7-^{3}H (E_1) in the presence of $NADPH_2$ at 37 for 3 h. Organic solvent extracts of the incubated tissue slices were separated by paper and silica gel thin layer chromatography. Radioactive testosterone (T) and estradiol-17β (E_2) were identified as the only conversion products of (Δ^4A) and (E_1). The mean rates of conversion of (Δ^4A) to (T) and (E_1) to (E_2) in normal and inflamed tissue samples were 3·5 and $7{\cdot}3 \times 10^{-7}$ μM/g/min in the males and 6·8 and $19{\cdot}4 \times 10^{-7}$ μM/g/min in the females. These results reveal the presence of 17β-ol-dehydrogenase in human gingival tissue. The significant increase in the biosynthesis of active sex steroids, (T) and (E_2), in inflamed as compared to normal gingiva of both sexes might be a factor in the aggravation of gingival inflammation due to the known hyperemic effects of these hormones. (Supported by USPH DE-03031-03.)