3. Biosynthesis

3A. Early stages in steroid hormone biosynthesis

51. Impairment of squalene epoxidation: a limiting step in cholesterol biosynthesis by human placenta

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We demonstrated recently *in vitro* the low but effective conversion of ^{3}H squalene to ^{3}H lanosterol by the microsomes of human placenta. The aim of the work is to determine why the epoxidase cyclase activity is markedly lower with placental microsomes than with hepatic. We have observed that with (1-^{14}C) oxydo-2,3 squalene as substrate, the conversion of this precursor to polycyclic triterpenes by human placental microsomes is raised up to 25%, a level comparable with that obtained in the same conditions with hepatic microsomes (30%). Thus we suggested that the rate limiting step in squalene cyclization in the placenta could be the aerobic step of squalene epoxidation. Since this metabolic blockage can be suppressed by hepatic cytosol containing squalene carrier protein (SCP), it could be related to a lack of SCP in the placental cytosol. Nevertheless, we could characterize in the placental cytosol, by gel filtration, a "SCP like" fraction with a limited binding capacity. Though having some characteristics identical with the hepatic SCP (electrophoretic mobility, filtration behaviour, polymerisation in the presence of squalene), the placental "SCP-like" fraction is thermosensitive (abolition of the limited binding capacity). Thus the low level of epoxidase cyclase activity of human placental microsomes could be related to a failure of the placental SCP to activate the aerobiotic epoxidation, rather than to a lack in squalene binding capacity or to a defect in the microsomal enzymatic system itself.

52. Synthesis and adrenocortical conversion of 20β-hydroperoxycholest-5-en-3β-ol-22-one

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Earlier studies on the adrenocortical metabolism of 20α-hydroperoxycholesterol suggested that a 20α-hydroperoxide —20α,22R-diol rearrangement may be involved as an intermediate step in the biosynthesis of pregnenolone. In seeking to obtain further information on the role and mechanism of the hydroperoxide rearrangement we explored various routes for the synthesis of 20-hydroperoxysterols. Oxygenation of 22-ketocholesterol at −20° in a binary solvent mixture gave 20β-hydroperoxycholest-5-en-3β-ol-22-one (I). The configuration at the 20-position was assigned upon reduction of the 20β-hydroperoxide group and comparison of the chemicophysical properties of the 3β,20β-dihydroxycholest-5-en-22-one with the known 20α-isomer. Thermal decomposition of *I* followed a similar pattern as that observed for 20α-hydroperoxycholesterol: hydroperoxide reduction to yield the 20β-hydroxy analog, C20–C22 bond cleavage to yield pregnenolone and cleavage of the C17–C20 bond to yield androstene products. Incubation of *I* with acetone dried adrenocortex mitochondria in phosphate buffer without added NADPH in an atmosphere of air or nitrogen resulted in the rapid formation of a single polar product which was obtained in cristalline form and identified as 3β,20β-dihydroxy-23,24-bisnorcholenic acid. Formation of the acid is suggested to proceed via an intramolecular hydroperoxide rearrangement in analogy with the enzymic conversion of 20α-hydroperoxycholesterol. The confinement of such reactions to the C-20-position of sterols may be viewed as further evidence for the existence of a transitory hydroperoxide-diol species as an intermediate in the biosynthesis of pregnenolone.

53. Mechanism of cholesterol side-chain cleavage in bovine adrenal cortex mitochondria

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Δ^{20-22}-Cholesterol (cholesta-5,20(22)-dien-3β-ol) (Δ^{20-22}) earlier described as a very poor substrate, was even faster converted into pregnenolone than 22R-OH-cholesterol (22R). The discrepancy is caused by small amounts of Δ^{17-20}-cholesterol (I) and Δ^{20-21}-cholesterol in the crude preparation. (I) especially proved to be a powerful inhibitor of cholesterol side-chain cleavage (CSCC). During the conversion of 20α-OH-cholesterol (20α) and 22R into pregnenolone 20α, 22R-di-OH-cholesterol (20α,22R) was formed as an intermediate. Its identity was confirmed by GC–MS. Both 20α and 22R used 2 mol O_2 per mol sterol substrate during the conversion to pregnenolone and isocaproaldehyde, while 20α,22R used 1 mol O_2. In short term incubations (20 min) only isocaproaldehyde was formed. The acid could be detected by GC in long term incubations (≥ 5 h) only. In the presence of 90% CO: 10% O_2 both 20α and 22R were almost quantitatively converted into 20α,22R. It is therefore improbable that a 20α- or 22R-hydroxylase is involved in the biosynthesis of 20α,22R. We propose the following mechanism: cholesterol → Δ^{20-22} → 20–22 cyclic peroxide → 20α,22R → pregnenolone + isocaproaldehyde. 20α and 22R will split off H_2O to form Δ^{20-22}.

54. Effects of aminoglutethimide on the side-chain cleavage of hydroxylated sterols; an experimental approach to congenital lipoid adrenal hyperplasia

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Congenital lipoid adrenal hyperplasia (CLAH) is an almost always fatal inborn error of cholesterol side-chain cleavage (CSCC) afflicting newborn children. With aminoglutethimide (AG), an inhibitor of CSCC, a disorder resembling CLAH can be induced in animals. The influence of AG on the CSCC was investigated *in vitro*. Intact bovine adrenal cortex mitochondria supported by malate were used. 3β-HSD was blocked with cyanoketone. In the absence of AG, side-chain cleavage of Δ^{20-22} cholesterol (Δ^{20-22}),