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}),

20α-OH-cholesterol (20α) and 22R-OH-cholesterol (22R) yielded pregnenolone + isocaproaldehyde. 25-OH-cholesterol (25-OH) formed pregnenolone + malonic dialdehyde + acetone. AG (40 μg/ml) fully blocked pregnenolone formation from cholesterol and 25-OH, while side-chain cleavage of Δ^{20-22}, 20α, and 22R was only partially inhibited. AG therefore exerts its main action on the reaction cholesterol → Δ^{20-22}. It is highly probable, that in CLAH *this* step is blocked. 25-OH in the presence of AG yields mainly 3β-OH-cholenic aldehyde (CA) + acetone while 20α partially yielded 20α,25-di-OH-cholesterol which was slowly converted into 20-hydroxylated CA. Isolated rat adrenal cells (stimulated with 1 mU ACTH/ml) were incubated with AG (20 μg/ml). Addition of 25-OH partially inhibited corticosterone production. Without AG,25-OH has a stimulating effect. We propose the hypothesis, that abnormal compounds like CA are responsible for the severity of CLAH.

55. On the unique status of cholesterol 20α-hydroperoxide in steroid metabolism

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Our prior demonstration of the rearrangement of cholesterol 20α-hydroperoxide to cholest-5-ene-3β,20α,22R-triol by bovine adrenal cortex mitochondria suggested the intermediacy of the 20α-hydroperoxide in pregnenolone biosynthesis from cholesterol. Additional studies of C_{27}-, C_{21}-, and C_{18}-hydroperoxide metabolism in mammalian, plant, and microbial systems failed to provide other examples of the hydroperoxide-diol rearrangement, reduction to the corresponding alcohol being commonly encountered. Formation of the 20α-hydroperoxide by rat adrenals and of cholesterol 7α- and 7β-hydroperoxides by rat liver has been observed, but enzymic hydroperoxide formation is not readily distinguished from nonenzymic peroxidation. Ethyl linoleate appears to stimulate 20α-hydroperoxide formation in rat adrenal incubations and markedly stimulates 7α- and 7β-hydroperoxide formation in incubations of soybean lipoxygenase or rat liver microsomes. The status of cholesterol 20α-hydroperoxide is unique as regards its metabolic rearrangement to a vicinal diol implicated in steroid hormone biosynthesis. (Supported by Robert A. Welch Foundation and U.S. Public Health Service Grant HL-10160).

56. Cholesterol side chain cleavage in microsomes and mitochondria from corpora lutea

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Adrenals effect cholestrol side chain cleavage (SCSS) only in mitochondria. This is not true in corpora lutea (CL) but luteal microsomal fractions have been little investigated. CL from pigs, sheep or cows were homogenized; nuclear, mitochondrial, microsomal and cytosol fractions were prepared by ultracentrifugation. Fractions were incubated for up to one hour in the presence of malate or succinate and an NADPH generating system, and cholesterol, pregnenolone and progesterone were determined by gas-liquid chromatography. CSCC activity was confined to microsomal and mitochondrial fractions and the specific activities (μg progesterone/mg protein) of the CSCC complex did not differ significantly between mitochondria and microsomes for any species. Under our incubation conditions, progesterone was produced rather than pregnenolone, regardless of cell fraction or species. Some mitochondrial preparations were examined in a 10–55% sucrose gradient using an MSE HS zonal rotor. Mitochondria were homogeneous in size; protein concentration, cytochrome C oxidase activity and CSCC activity were well correlated. We conclude that, in luteal cells, mitchondria and endoplasmic reticulum are equally important in CSCC and, if LH controls progesterone biosynthesis, both fractions should be responsive to the ultimate effector of the gonadotrophin.

3B 1. Steroid biosynthesis: Adrenal Cortex—I

57. Alternative pathways of corticosteroid synthesis in rat adrenals

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After incubation of rat adrenal quarters with ^{3}H-acetate, specific radioactivities of cholesterol, pregnenolone and progesterone were 10–100 times lower than those of 11-desoxycorticosterone (DOC) and corticosterone (B). ACTH decreased specific radioactivities of cholesterol by factors of 3–8, but it did not alter those of B and it increased those of DOC 2–3 fold. It seems to be unlikely, therefore, that ^{3}H-acetate had been incorporated into DOC and B *via* cholesterol, pregnenolone and progesterone. Specific radioactivities of cholesterol analyzed separately in mitochondria and in the remaining cell fraction were identical. This does not support the hypothesis that only a small pool of highly labelled cholesterol (which should be expected within the mitochondria) serves as steroid precursor. 21-OH-pregnenolone, the only alternative to progesterone as direct precursor of DOC, was 30–50 (control) and 3–9 (ACTH) times higher in specific radioactivity than DOC and B. Under the influence of "triparanol" (1-(p-diethylaminoethoxyphenyl)-1-(p-tolyl)-2-(p-chlorophenyl)-ethanol) which is known to inhibit the step "desmosterol-cholesterol", specific radioactivities of cholesterol decreased to $\frac{1}{10}$ of the control values. In contrast, there were only slight alterations in the specific radioactivities of 21-OH-pregnenolone, DOC and B. These data strongly suggest that in rat adrenals DOC and B can be synthesized from acetate *via* alternative pathways not including cholesterol, pregnenolone and progesterone as intermediates, in which 21-OH-pregnenolone may be the direct precursor of DOC.

58. Reciprocal interactions of progesterone and 17α-hydroxyprogesterone as exogenous substrates of rat adrenal 21-hydroxylase

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Due to the small concentration and activity of 17α-hydroxylase present in the rat adrenal, the main corticoids secreated in the rat are DOC, B_k, A_k, 18-OH-DOC and aldosterone, formed directly from progesterone(I). Because of the limited amounts of 17α-OH-progesterone (II) available, the biosynthesis of S_R, F_k and E_k is restricted. Since