

20 $\alpha$ -OH-cholesterol (20 $\alpha$ ) and 22R-OH-cholesterol (22R) yielded pregnenolone + isocaproaldehyde. 25-OH-cholesterol (25-OH) formed pregnenolone + malonic dialdehyde + acetone. AG (40  $\mu$ g/ml) fully blocked pregnenolone formation from cholesterol and 25-OH, while side-chain cleavage of  $\Delta^{20-22}$ , 20 $\alpha$ , and 22R was only partially inhibited. AG therefore exerts its main action on the reaction cholesterol  $\rightarrow$   $\Delta^{20-22}$ . It is highly probable, that in CLAH *this* step is blocked. 25-OH in the presence of AG yields mainly 3 $\beta$ -OH-cholesterol (CA) + acetone while 20 $\alpha$  partially yielded 20 $\alpha$ ,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  $\mu$ g/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 20 $\alpha$ -hydroperoxide in steroid metabolism

SMITH, L. L. and TENG, J. I., Division of Biochemistry, University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.

Our prior demonstration of the rearrangement of cholesterol 20 $\alpha$ -hydroperoxide to cholest-5-ene-3 $\beta$ ,20 $\alpha$ ,22R-triol by bovine adrenal cortex mitochondria suggested the intermediacy of the 20 $\alpha$ -hydroperoxide in pregnenolone biosynthesis from cholesterol. Additional studies of C<sub>27</sub>, C<sub>21</sub>, and C<sub>18</sub>-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 20 $\alpha$ -hydroperoxide by rat adrenals and of cholesterol 7 $\alpha$ - and 7 $\beta$ -hydroperoxides by rat liver has been observed, but enzymic hydroperoxide formation is not readily distinguished from nonenzymic peroxidation. Ethyl linoleate appears to stimulate 20 $\alpha$ -hydroperoxide formation in rat adrenal incubations and markedly stimulates 7 $\alpha$ - and 7 $\beta$ -hydroperoxide formation in incubations of soybean lipoxygenase or rat liver microsomes. The status of cholesterol 20 $\alpha$ -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

COOK, B., STRUTHERS, A. D., DENHOLM, R. B. and TAYLOR, PATRICIA D., University Department of Steroid Biochemistry, Royal Infirmary, Glasgow, G4 0SF, Scotland

Adrenals effect cholesterol 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

( $\mu$ g 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, mitochondria 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

LOMMER, D., DIEDRICHSSEN, G. and SINTERHAUF, K., 1 Department of Medicine, University of Mainz, D-65 Mainz, W-Germany

After incubation of rat adrenal quarters with <sup>3</sup>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 <sup>3</sup>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 17 $\alpha$ -hydroxyprogesterone as exogenous substrates of rat adrenal 21-hydroxylase

ORTA, Z. DE M. and DOMINGUEZ, O. V., Division of Biochemistry, Department of Scientific Research (IMSS), Mexico City, D.F., Mexico

Due to the small concentration and activity of 17 $\alpha$ -hydroxylase present in the rat adrenal, the main corticoids secreted in the rat are DOC, B<sub>k</sub>, A<sub>k</sub>, 18-OH-DOC and aldosterone, formed directly from progesterone(I). Because of the limited amounts of 17 $\alpha$ -OH-progesterone (II) available, the biosynthesis of S<sub>R</sub>, F<sub>k</sub> and E<sub>k</sub> is restricted. Since