CONVERSION OF 20-α-HYDROXYCHOLESTEROL TO PREGNENOLONE*

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20-α-Hydroxycholesterol was first reported by Solomon et al. (1956) to be an intermediate in the conversion of cholesterol to the adrenal steroids. Recently, Shimizu et al. (1960) have reported the formation of labeled isocaproic acid when 20-α-hydroxycholesterol-26-C^{14} was incubated with soluble adrenal extract in the presence of ATP, Mg^{++}, DPN^{+}, and fumarate. We wish to report in this communication the requirement of a reduced pyridine nucleotide and multiple enzyme fractions for the conversion of 20-α-hydroxycholesterol-7-α-T to T-labeled pregnenolone.

Materials and Methods: - Two soluble enzyme fractions A and B were prepared from acetone powder of beef adrenal mitochondria as previously described (Constantopoulos and Tchen, 1961). 20-α-hydroxycholesterol-7-α-T of specific activity 5×10^5 c. p. m./mg. was synthesized from pregnenolone-7-α-T (purchased from New England Nuclear Corp.) (Petrow and Stuart-Webb, 1956). Separation of sterols was carried out by paper chromatography according to Zaffaroni and Burton (1956).

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Results and Discussion: - We have previously reported that the conversion of cholesterol to pregnenolone and isocaproic acid requires two protein fractions and either TPNH or DPNH (Constantopoulos and Tchen, 1961). Experiments with 20-α-hydroxycholesterol revealed that the same requirements must be met for its conversion to pregnenolone. This is summarized in Table I.

TABLE I

Cofactor	c. p. m. pregnenolone	c.p.m polar compounds
None	-	-
DPNH	35,000	5,000
TPNH	35,000	5,000
DPN^+	-	-
TPN^+	-	-
Enzyme Fractions		
A	-	-
B	-	-
A + B	35,000	5,000

50,000 c. p. m. of 20-α-hydroxycholesterol was incubated with enzyme fractions and cofactors as previously described (Constantopoulos and Tchen, 1961) with the modification that increased amounts of enzymes, corresponding to 6 gm. of adrenal cortex, were used.

We have also reported that the cleavage of the side-chain of cholesterol leads to the formation of isocaproic aldehyde which is subsequently oxidized to isocaproic acid (Constantopoulos and Tchen, 1961). With 20-α-hydroxycholesterol as an intermediate and pregnenolone and isocaproic aldehyde as the products, the cleavage of the side-chain of cholesterol by adrenal extracts may be expected to follow one of the two path-

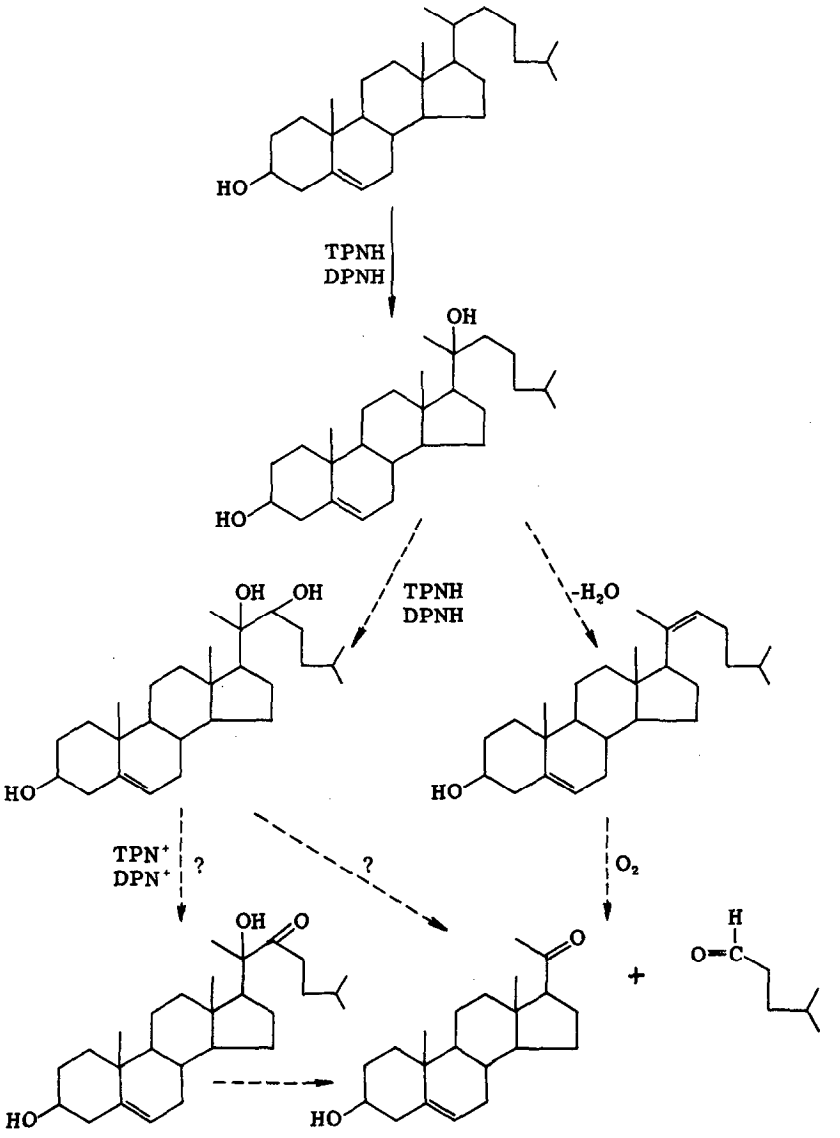

FIGURE I

ways illustrated in Figure I. The pathway on the right, with the formation of a double bond between carbon atoms 20 and 22 and its cleavage by molecular oxygen, should not require the addition of pyridine nucleotides (see review by Mason, 1957). The observed requirement of a reduced

pyridine nucleotide, which is characteristic for hydroxylation reactions, strongly suggests that 20, 22-dihydroxy- and 20-hydroxy-22-keto-cholesterol are involved in the biogenesis of pregnenolone.

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