PRELIMINARY NOTES

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Relation of cholesterol synthesis and NADPH oxidation by microsomal electron transport system involving P-450

With microsomes from liver, it has been found that the hydroxylation of various drugs such as aminopyrine or aniline is inhibited by carbon monoxide in the same manner as is the oxidation of NADPH by an electron transport system involving P-450[1-3], which is capable of binding carbon monoxide[4]. In addition, it has been shown that liver microsomes, prepared from rats which had been treated with phenobarbital *in vivo*, exhibit a parallel increase in the content of components of the electron transport system and in the activities of oxidative demethylation[5]. From these findings, it was suggested that the electron transport system was functional in the hydroxylation of the drugs, and this suggestion has been currently accepted. The drugs reported hitherto are apparently unphysiological, nevertheless the requirement of the electron transport system for the hydroxylation of a naturally occurring substance can be considered in view of the fact that cholesterol is synthesized by a series of reactions, including certain microsomal ones, which require both NADPH and molecular oxygen[6].

The present paper deals with studies on the synthesis of cholesterol from either acetate or mevalonate. It was found that cholesterol synthesis was faster with liver preparations from phenobarbital-administered rats than with those from control rats, and was inhibited by carbon monoxide.

Male rats of the Sprague–Dawley strain were divided into two groups. One group were for control and the others were fed with a diet containing 0.1 % phenobarbital for a week. The assay of cholesterol synthesis was carried out according to the method of BUCHER *et al.*[7]. The livers from the rats of the two groups were separately homogenized in 2.5 vol. of the medium of pH 7.4 containing 0.004 M magnesium chloride, 0.03 M nicotinamide, 0.125 M sucrose, 0.001 M disodium EDTA, 0.01 M glutathione and 0.1 M potassium phosphate buffer, followed by centrifuging at 10000 × *g* for 10 min. The resulting supernatant was used as a liver preparation for the assay. The components of the reaction mixture were as follows; in a total volume of 2.5 ml, 2.0 ml of the liver preparation, 0.0016 M NAD, 0.016 M potassium fructose 1,6-diphosphate, and 0.004 M [1-^{14}C]acetic acid or [2-^{14}C]mevalonic acid (0.1 μC). The reactions were carried out at 37° for 1 and 2 h with mevalonate and acetate, respectively, and then stopped by adding 2.5 ml of 15 % potassium hydroxide in 95 % ethanol. After being heated at 60° for 1 h, the resulting mixture was supplemented with 2 mg of carrier cholesterol, and extracted with 20 ml of petroleum ether. The extraction was repeated two times, and the extracts were mixed and evaporated to dryness. The resulting residue was dissolved in 10 ml of acetone–ether (1:1, v/v). To the solution was added 2.5 ml of 1 % digitonin in 50 % ethanol in order to precipitate cholesterol as its digitonide. The resulting precipitate was washed successively

TABLE I

EFFECT OF CARBON MONOXIDE ON SYNTHESIS OF CHOLESTEROL WITH ACETATE AND WITH MEVALONATE

Gas phase	*^{14}C incorporation into cholesterol*			
	with [^{14}C]acetate		*with [^{14}C]mevalonate*	
	*Counts/min per mg**	%	*Counts/min per mg**	%
95% N_2, 5% O_2	87	(100)	1470	(100)
95% CO, 5% O_2	69	79	730	50

* Total counts incorporated per endogenous cholesterol + carrier cholesterol (2 mg) in each incubation mixture.

TABLE II

EFFECT OF PHENOBARBITAL ADMINISTRATION ON SYNTHESIS OF CHOLESTEROL WITH ACETATE AND WITH MEVALONATE

Rats for liver preparations	*^{14}C incorporation into cholesterol*			
	with [^{14}C]acetate		*with [^{14}C]mevalonate*	
	*Counts/min per mg**	%	*Counts/min per mg**	%
Control	102	(100)	2670	(100)
Phenobarbital-administered	120	118	12250	460

* Total counts incorporated per endogenous cholesterol + carrier cholesterol (2 mg) in each incubation mixture.

with acetone, acetone–ether (1:1, v/v), and ether. The washed cholesterol digitonide was dissolved in an appropriate volume of methanol. Radioactivity was measured on an aliquot of the solution by a scintillation counter. The quantitative determination of cholesterol was carried out according to the method of ZAK *et al.*[8].

When either [^{14}C]acetate or [^{14}C]mevalonate was added to the reaction mixture containing the liver preparation, the radioactivity was incorporated into the cholesterol synthesized, provided that the reaction was carried out under aerobic conditions. The ^{14}C incorporations (cholesterol synthesis) with acetate and with mevalonate were reduced in rate when the reactions were carried out under gas phases containing carbon monoxide: at 95% CO, the rate was 79% with acetate and 50% with mevalonate (Table I). Since the liver preparations used contained microsomes, it seems rational to speculate that the blocking of the microsomal electron transport system due to the binding of the P-450 with carbon monoxide is responsible for the carbon monoxide inhibition of the cholesterol synthesis.

In good accordance with the findings of ORRENIUS, ERICSSON AND ERNSTER[5], it was found that with the liver preparations used, components of the microsomal electron transport system catalyzing the oxidation of NADPH through P-450 by molecular oxygen were remarkably increased in amount when the rats were fed with a diet containing phenobarbital. In addition, it was found that the cholesterol synthesis with acetate and with mevalonate were faster when the reactions were carried out

with the liver preparations from the phenobarbital-administered rats than with those from the control ones; the increase in rate was much more significant with mevalonate than with acetate (Table II). These data also suggest an intimate relation between the cholesterol synthesis and the microsomal electron transport system. BUCHER *et al.*[7] reported that in the cholesterol synthesis with acetate, a rate-limiting process was the formation of mevalonate. It is probable, therefore, that a reaction involved in the synthesis of cholesterol from mevalonate depends upon the NADPH oxidation catalyzed by the electron transport system. The substrate for the reaction should be a physiological one.

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Effect of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone on chlorophyll fluorescence and photosynthesis

According to current theory[1] relating chlorophyll fluorescence to photosynthetic electron transport, fluorescence is emitted from photosystem II and becomes stronger as the acceptor for system II becomes more reduced. Increased fluorescence after 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) addition is due to blocking of a step between systems I and II—reoxidation of the system II acceptor being prevented. DCMU would not affect photosystem I or its associated dark reactions (including phosphorylation). The theory predicts that uncouplers of phosphorylation will not decrease the strong fluorescence of DCMU-inhibited cells, since they act on the system I side of the DCMU-blocked step and cannot increase the supply of acceptor for system II. In fact, this paper shows that the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) more than halves the fluorescence of DCMU-inhibited cells.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.