THE EFFECT OF EXOGENOUS CHOLESTEROL ON THE SYNTHESIS IN VIVO OF CHOLESTEROL, UBIQUINONE, AND SQUALENE IN RAT LIVER

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Received February 8, 1967

It has been repeatedly shown that feeding of cholesterol results in a marked depression of hepatic cholesterol synthesis (Gould, 1951; Tomkins et al., 1953; Langdon and Bloch, 1953; Frantz et al., 1954; Siperstein and Fagan, 1966). This was demonstrated to be due to a sensitive feedback inhibition operating to control the enzyme β-hydroxy-β-methyl glutaryl CoA reductase thus limiting the synthesis of mevalonate (Siperstein and Fagan, 1966).

The isoprenoid side chain of ubiquinone is synthesized from acetate via mevalonate. Both acetate and mevalonate have been shown to be incorporated into the side chain of ubiquinone in animals (Gloor and Wiss, 1959; Olson and Dialameh, 1960). Cholesterol and ubiquinone are thus synthesized through a common biosynthetic pathway involving mevalonate. Although the exact point at which branching takes place is unknown, it is likely that it occurs at the isoprenyl pyrophosphate level. If cholesterol inhibits its own synthesis by depressing the activity of HMG-CoA reductase, it should also inhibit the synthesis of ubiquinone unless additional control points exist. It is the purpose

of this communication to report the results obtained in a study of synthesis in vivo of cholesterol, ubiquinone and squalene in cholesterol-fed rats. It is shown here that under the conditions of severe inhibition of hepatic cholesterol synthesis the formation of ubiquinone is unaffected or even slightly increased. The present results suggest the existence of a secondary control point for the cholesterol biosynthesis at the cyclization of squalene.

Experimental

Paper chromatographically pure acetate-1-C^{14} and mevalonate-2-C^{14} were obtained from New England Nuclear and Nuclear Chicago respectively. They were used without any further purification. Male Sprague Dawley rats (weighing between 200 and 300 g.) maintained on normal synthetic diet were divided into two groups. One group received normal diet while the other received same diet but having cholesterol at 5% level. The feeding was continued for 4 days after which the rats were given either acetate-1-C^{14}(S.A. 53.0 mc/mmole) or with mevalonate-2-C^{14} (S.A. 5.03 mc/mmole) and killed after 1 hr. The livers were removed quickly into ice cold beakers, weighed and analyzed for cholesterol, ubiquinone and squalene and for the radioactivity in those fractions. Saponification of the tissues and the chromatography of unsaponifiable fraction on deactivated alumina column to separate hydrocarbon, ubiquinone and cholesterol fractions were carried out according to Olson et al. (1965). Cholesterol was precipitated as the digitonide from the unsaponifiable lipids and estimated by Liebermann-Burchard reaction. Squalene, present in the hydrocarbon fraction, was isolated by thin layer chromatography on silica gel G (developed with petroleum ether) and estimated according to the method of Rothblat et al. (1962).

Ubiquinone was purified by reverse phase thin layer chromatography on silica gel G (5% paraffin impregnated, developed with acetone-water, 9:1) and estimated spectrophotometrically. The radioactivity in all the fractions was measured in a liquid scintillation spectrometer.

Results and Discussion

Table 1 shows the results when acetate-1-C^{14} was used as the tracer to follow the hepatic synthesis of cholesterol, ubiquinone and squalene in normal and cholesterol-fed rats. It can be seen that exogenous cholesterol markedly inhibited endogenous sterol synthesis, an observation which has been reported by several workers earlier. As was expected, the pool size and incorporation of label into squalene was also reduced. These changes appear to be due to limiting amounts of mevalonate (Siperstein and Fagan, 1966). However, in spite of this feedback inhibition at mevalonate level, the pool size and the synthesis of ubiquinone, as judged by the incorporation of label from radioactive acetate are unaffected in cholesterol-fed rats. Although there is a slight decrease in the total concentration of ubiquinone (see Table 2 also) this difference was found to be statistically insignificant. It must be remembered, however, that the usual biosynthetic rate of ubiquinone in the rat is 2-3% of that of cholesterol (Olson et al. 1965). Therefore, these results demonstrate that the small amount of mevalonate synthesized in cholesterol-fed rats is very efficiently used for the assembly of the side chain of ubiquinone, and the alkylation proceeds at a normal rate. These results also suggest the existence of a secondary control point for cholesterol biosynthesis probably after the branch point in order to divert isoprenoid carbon towards ubiquinone.

Table 1

THE INCORPORATION OF ACETATE-1-C^{14} INTO CHOLESTEROL, UBIQUINONE AND SQUALENE OF RAT LIVER

Status of Rat	Liver wt. g.	Cholesterol mg.	Ubiquinone µg.	Squalene µg.
Normal	11.8	24.8 (35,120)	980 (374)	445 (2024)
Cholesterol-fed	11.8	57.5 (4328)	876 (428)	220 (912)

Each rat was injected intraperitoneally with 50 µc of acetate-1-C^{14} and killed after 1 hr. Values are expressed per whole liver and represent averages from five or more rats. Values in the parentheses indicate the radioactivity in that fraction as d.p.m.

To test the above possibility more rigorously, additional experiments were carried out with mevalonate-2-C^{14} as the tracer instead of acetate-1-C^{14}. Table 2 shows the results of these experiments. The marked inhibition in the hepatic cholesterol synthesis observed in cholesterol-fed rats with acetate-1-C^{14} was also detected with mevalonate-2-C^{14}. The specific activity of cholesterol decreased 9 fold in these rats and radioactivity accumulated in the ubiquinone and squalene fractions. Their specific activities were increased by 3 and 11 fold respectively. These results strongly suggest that the cyclization of squalene is also reduced by cholesterol feeding and as a result of this block, isoprenoid carbon is more efficiently used for the synthesis of ubiquinone. It should be mentioned here that Lee and Draper (1964) earlier reported that the incorporation of 2-C^{14}-mevalonate into hepatic ubiquinone in cholesterol-fed rats was very high as compared to normals. However, they found the

ubiquinone concentrations in such rats to be significantly lowered; our present results do not confirm this finding.

Table 2

THE INCORPORATION OF MEVALONATE-2-C^{14} INTO CHOLESTEROL, UBIQUINONE AND SQUALENE OF RAT LIVER

Status of Rat	Liver wt. g.	Cholesterol mg.	Ubiquinone µg.	Squalene µg.
Normal	11.2	30.7 (454,340)	904 (3532)	493 (118,820)
Cholesterol-fed	12.8	86.5 (140,920)	736 (10,208)	255 (668,650)

Each rat was injected intraperitoneally with 5 µc of 2-C^{14}-mevalonic acid lactone and killed after 1 hr. Values are expressed per whole liver and represent averages from two rats. Values in the parentheses indicate the radioactivity in that fraction as d.p.m.

From studies of rates of conversion of metabolites to cholesterol in homogenates from cholesterol-fed rats, Gould and Swyryd (1966) recently concluded that at least two more sites of control for hepatic cholesterol synthesis exist between mevalonate and squalene. Although it is possible that several enzymes in the biosynthesis of cholesterol can be regulated, the results in Table 2 show unequivocally that the cyclization of squalene is a second major control point. Studies in this laboratory with rats in various states of vitamin A nutriture from deficiency to excess have shown that vitamin A also regulates the squalene cyclization step in liver (Olson, 1966; Subba Rao and Olson, 1967). Thus, it appears that the enzyme squalene cyclohydroxylase, which catalyzes the conversion of squalene to lanosterol plays an important role in regulating the metabolism of isoprenoid

compounds in rat liver. Experiments to study the activity of this enzyme *in vitro* in various nutritional conditions are in progress.

Acknowledgment. We thank Jon Miller for assistance in the performance of some of these experiments. This work was supported-in-part by grant AM-09992 from the U.S. P.H.S.

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