STIMULATION OF HEPATIC CHOLESTEROL BIOSYNTHESIS BY OLEIC ACID[1]

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SUMMARY

Livers from normal fed male rats were perfused in vitro with an erythrocyte-free, bloodless medium containing serum albumin (3%), and glucose (100 mg %). Oleic acid (663 μmoles) bound to albumin, or albumin alone, was infused at a constant rate. Biosynthesis of cholesterol was evaluated by incorporation of radioactivity from ^3H_2O. Oleic acid stimulated output of cholesterol (1.60 ± 0.08 SEM vs 1.18 ± 0.04 μmoles/g) but did not change the concentration of cholesterol in the liver or hepatic microsomes. Incorporation of ^3H into cholesterol was stimulated by oleate; dpm per μmole cholesterol/dpm per μg atom H was 3.94 ± 0.33, 3.46 ± 0.32, and 4.46 ± 0.37 in the total cholesterol of liver, perfusate, and microsomes, respectively, when oleate was infused. Corresponding values when oleate was not infused were 1.71 ± 0.23, 1.62 ± 0.20, and 2.09 ± 0.26, respectively ($P<0.001$ in all cases). It is suggested that the stimulation of biosynthesis of cholesterol by oleate results from the obligatory requirement of cholesterol, as a moiety of the very low density lipoprotein, for the secretion of triglyceride by the liver.

INTRODUCTION

A very low density lipoprotein (VLDL)* is secreted by the liver and is the form in which triglyceride is transported from liver to other tissues for oxidation or storage, dependent on metabolic need (20). The VLDL is a vehicle for the dispersal and transport of triglyceride in the aqueous environment of the blood and contains, in addition to triglyceride, considerable quantities of phospholipid and cholesterol, which, because of their relatively polar characteristics in comparison to triglyceride, serve to form a stable structure; the

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*Abbreviations used: VLDL, very low density lipoprotein; TG, triglyceride; PL, phospholipid; C, cholesterol.

triglyceride of the VLDL, however, is the metabolically important substrate whose transport through the blood is required. It has been observed that the hepatic secretion of phospholipid and cholesterol into the VLDL is proportional to the output of TG (9), and that the molar ratio of TG : PL : C in the VLDL is relatively constant under specific conditions (9,26), although the ratio may vary, for example, with the quantity and structure of the free fatty acid perfusing the liver (11). Since cholesterol and phospholipid are essential moieties of the VLDL and therefore obligatory for the secretion of triglyceride by the liver, it may be expected that any stimulant or inhibitor of output of triglyceride should have a similar effect on the secretion of cholesterol and phospholipid. Just as the output of triglyceride by the liver is stimulated by exogenous fatty acids (11,14,27), so are also the output of cholesterol and phospholipid in the VLDL (11). It is a logical extension of these observations to postulate that, if cholesterol, for example, is necessary for the secretion of triglyceride, then free fatty acids may stimulate the biosynthesis as well as the output of cholesterol by the liver. The purpose of this investigation was to evaluate that postulate. Accordingly, the effects of oleic acid on the output of triglyceride and cholesterol, and on the biosynthesis of cholesterol, using $^{3}H_2O$ as a precursor, were studied with the isolated perfused rat liver. It seems most probable from the data reported here, that free fatty acids are important regulators of hepatic cholesterogenesis.

METHODS

Livers for perfusion were obtained from normal male rats (210-230 g body weight) which had been maintained on a laboratory ration (Purina Rat Chow) and water ad libitum. The rat livers were isolated surgically (8) and were perfused in vitro using the apparatus described previously (10). In these experiments, the rate of flow of perfusate, at 20 cm hydrostatic pressure, was maintained at 60-75 ml/min.* The livers were perfused initially for a period of 20 minutes with a medium consisting of 70 ml Krebs-Henseleit bicarbonate

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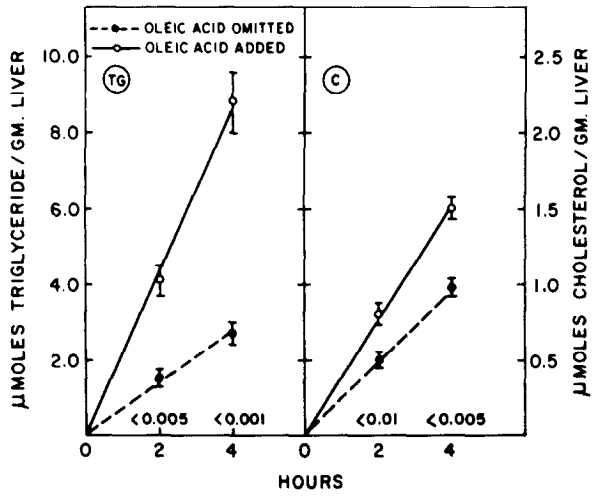

Figure 1

Title: Output of Triglyceride and Cholesterol by the Rat Liver Perfused with a Blood-Free Medium.

Legend: The data presented are mean values ± S.E.M. for the cumulative net output of triglyceride and total cholesterol respectively. The significance of the differences between means, derived from a two-tailed table of values for "t", is included in the figure. Liver weight (wet) at the termination of perfusion was 8.62 ± 0.25 g and 8.59 ± 0.28 for the groups perfused in the absence or presence of added oleate, respectively; corresponding values for secretion of bile were 1.95 ± 0.16 and 2.08 ± 0.10 ml per 4 hours. ^3H_2O was not included in the perfusate in these experiments.

buffer, pH 7.4 (23), 100 mg/100 ml of glucose and 3 g/100 ml of purified bovine serum albumin (11). Erythrocytes, blood, or serum was deliberately omitted from the perfusate to avoid rapid equilibration of newly synthesized cholesterol with the cholesterol in erythrocytes or plasma lipoproteins (4,7,13,17,18). After the 20 minute period of equilibration, a complex of bovine serum albumin and oleic acid (708 μmoles oleate/2.5 g albumin/50 ml in 0.9% NaCl) was infused (11). The complex was infused into the perfusate at a constant rate (11.7 ml/hr; 166 μmoles/hr, during the 4 hour experimental period). Albumin was infused alone in control experiments. For studies of cholesterol biosynthesis, ^3H_2O was added to the basal medium and to the serum albumin-oleate complex to provide 4000 DPM/μg atom hydrogen in the water (13.63 mc/70 ml original medium, and 9.18 mc/50 ml albumin-oleate complex, or albumin alone). At the termination of the

Table 1

Stimulation of Hepatic Cholesterogenesis by Oleic Acid

GROUP	CHOLESTEROL[1]	RSA[2]	RTA[3]
I. Oleic Acid Omitted:			
A. Whole Liver (7)	5.04 ± 0.26	1.71 ± 0.23	8.08 ± 1.31
B. Microsomes (7)	44.96 ± 1.84	2.09 ± 0.26	91.56 ± 8.12
C. Perfusate (7)	1.18 ± 0.40	1.62 ± 0.20	1.94 ± 0.29
II. Oleic Acid Added:			
D. Whole Liver (5)	5.00 ± 0.20	3.94 ± 0.33	19.78 ± 1.84
E. Microsomes (5)	41.12 ± 4.31	4.46 ± 0.37	194.80 ± 22.71
F. Perfusate (5)	1.60 ± 0.08	3.46 ± 0.32	5.53 ± 0.63
Statistics: (P values, less than indicated values, were derived from a 2-tailed table for student's values for "t")			
A vs D	NS	<.001	<.001
B vs E	NS	<.001	<.001
C vs F	<.001	<.001	<.001

[1]Concentration of total cholesterol in the whole liver is expressed as μmoles/g liver, and in microsomes as μmoles/g protein. For perfusate, figures given are net output of total cholesterol/g liver/4 hours. All data are means ± standard error. Figures in parentheses indicate number of observations.

[2]RSA = Relative Specific Activity

$$= \frac{\text{DPM/}\mu\text{mole cholesterol}}{4000\ \text{DPM/}\mu\text{g atom H in } H_2O}$$; see reference 25

[3]RTA = Relative Total Activity = (RSA)(μmoles cholesterol/g liver or g protein)

experiment, the livers were perfused with 20 ml of ice-cold 0.9% NaCl, cleansed of adherent nonhepatic tissue, blotted, weighed, minced with 10 ml of ice-cold 0.3 M sucrose-0.2 M EDTA, and homogenized. The final volume of solution was adjusted to 3 ml/g liver, wet weight. Microsomes were isolated from the homogenate (22) and microsomal protein estimated (15). Lipids were extracted from

aliquots of liver homogenates, hepatic microsomes, and perfusate, with $CHCl_3$-CH_3OH (2:1, v/v), and separated into lipid classes by thin layer chromatography (11). After development of the plates and identification of the lipid fractions, the bands of neutral lipids were transferred to 10 x 120 mm glass columns and eluted with 10 ml $CHCl_3$. Aliquots of the appropriate extracts were analyzed for triglycerides (24). Portions of the extracts containing either cholesterol or cholesteryl esters were dried in vacuo and saponified with 10% KOH in 80% ethanol (19). Aliquots of the nonsaponifiable fraction were analyzed for cholesterol using the O-phthalaldehyde reagent (21,28). Radioactivity in the nonsaponifiable fraction was measured by liquid-scintillation counting in a Beckman CPM-100 counter using diluted Permafluor (Packard). The data reported here for total cholesterol are the sums of free and esterified cholesterol, measured separately.

Oleic acid was purchased from Nu-Chek-Prep. Bovine serum albumin (Fraction V, Pentex) was extracted with isooctane-glacial acetic acid (6), and with anhydrous methanol to remove phospholipid*, it was then dialyzed and lyophillized before use. Chemicals used were reagent grade and all solvents were redistilled from glass before use. Silica gel G plates, 250 μ thick, were purchased from Analtech, Inc. Rats were obtained from the Holtzman Company, Madison, Wisconsin. Tritiated water was obtained from New England Nuclear, and from ICN.

RESULTS AND DISCUSSION

The output of triglyceride and total cholesterol by the liver perfused in the absence of blood or erythrocytes can be seen in Figure 1. The rate of output of triglyceride and cholesterol was linear as a function of time during the experiment (figure 1). Addition of oleic acid to the medium resulted in an increase in output of triglyceride and cholesterol by the liver, in confirmation of previously published data (9), but did not result in any change of concentration of cholesterol within the liver (Table 1). The incorporation of tritium from ^3H_2O into cholesterol of liver and perfusate can be seen in Table 1.

*Wilcox, H. G., Dunn, G. D., and M. Heimberg, unpublished data.

More total radioactivity from tritium was incorporated into cholesterol of the liver, hepatic microsomes, and perfusate when oleate was infused than when the fatty acid was omitted. Furthermore, the specific activity of the cholesterol was increased more than 100% above the control as a result of infusion of oleic acid. The similarity of cholesterol relative specific activity in the liver and perfusate testifies to the rapid equilibration of newly synthesized cholesterol with pre-existing pools (4,13).

It is clear that oleic acid is a potent stimulant for both biosynthesis of cholesterol by the liver and secretion of cholesterol into the perfusate, and that the addition of the free fatty acid to the medium results in a net increase in the total quantity of cholesterol, as well as of triglyceride (14), in the liver plus perfusate. The stimulus for the increased synthesis of cholesterol presumably is the need to form and secrete a VLDL for the transport of triglyceride. It is probable that, in vivo, plasma free fatty acids, and therefore ultimately the dietary triglycerides from which plasma free fatty acids can be derived in man and animals (16,20), are important regulators of net output and de novo biosynthesis of cholesterol, and triglyceride (16), by the liver. It is known that dietary triglyceride can alter the plasma concentration of triglyceride, phospholipid, and cholesterol; the physiological mechanisms by which this is brought about may, in part, depend on the effects of free fatty acids on the secretion of the VLDL lipids by the liver. It is pertinent to these postulates that feeding of neutral fats (i.e., triglycerides) to animals has been reported to stimulate cholesterol biosynthesis by the liver (1,3,5,12); this effect, moreover, is accompanied by increased hepatic activity of hydroxymethylglutaryl coenzyme A reductase, E.C. 1.1.1.34 (2,5). The observation that free fatty acids can stimulate biosynthesis of cholesterol by the perfused liver in vitro (i.e., positive feedback) opens up the problem of control mechanisms to more direct attack. The molecular mechanisms by which oleate, and presumably other fatty acids, exert their action on biosynthesis of cholesterol remain to be determined, and are the directions for future investigations.

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