Regulation of rat hepatic cholesterol metabolism. Effects of lipoprotein composition on acyl coenzyme A:cholesterol acyltransferase in vivo and in the perfused liver and on hepatic cholesterol secretion[1]

Paul E. A. Van Zuiden,* Allen D. Cooper,[2] and Sandra K. Erickson[3]

Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305 and Department of Medicine, Brown University* and Gastroenterology Section, Veterans Administration Medical Center, Providence, RI 02908

Abstract Lipoproteins that are removed from the circulation by the liver can deliver both cholesterol and triglycerides to the hepatocyte. Relative proportions of these lipids may vary in lipoproteins and, thus, their uptake may have differing effects on cholesterol homeostasis. To study this, lipoproteins containing the same amounts of cholesterol but different amounts of triglyceride were administered to intact rats or to an isolated perfused rat liver. The responses of acyl coenzyme A:cholesterol acyltransferase (ACAT), very low density lipoprotein (VLDL) triglyceride and cholesterol secretion, and biliary cholesterol content were examined after 2 hr. Administration of triglyceride-rich chylomicrons (average triglyceride:cholesterol = 136.5 by mass) in vivo or their remnants (average triglyceride:cholesterol = 32.7 by mass) to the perfused liver resulted in an 80% decrease in ACAT activity. In the perfused liver system, VLDL cholesterol and triglyceride secretion was increased while biliary cholesterol content decreased. Administration of standard chylomicrons (average triglyceride:cholesterol = 33.9 by mass) or their remnants (average triglyceride:cholesterol = 11.4 by mass) lowered ACAT activity by 24% in vivo, but had no significant effect on any of the parameters measured in the perfused liver system. Administration of cholesterol-rich VLDL (average triglyceride:cholesterol = 0.47 by mass) in vivo increased ACAT activity 1.4-fold, but administration of their remnants (average triglyceride:cholesterol = 0.17 by mass) had little effect on any of the parameters measured in the perfused liver. Thus, the lipid composition of lipoproteins removed by the liver elicited acute responses by parameters important in the maintenance of hepatic cholesterol homeostasis. These responses reflected the net effects of both the cholesterol and the triglyceride contents of the particles. — **Van Zuiden, P. E. A., A. D. Cooper, and S. K. Erickson.** Regulation of rat hepatic cholesterol metabolism. Effects of lipoprotein composition on acyl coenzyme A:cholesterol acyltransferase in vivo and in the perfused liver and on hepatic cholesterol secretion. *J. Lipid Res.* 1987. **28:** 930–940.

Supplementary key words chylomicrons • cholesterol-rich very low density lipoproteins • lipoprotein remnants • very low density lipoproteins • bile

The liver plays a major role in the regulation of lipoprotein and biliary metabolism. Changes in lipoprotein metabolism can be induced by perturbations in the rates of synthesis and secretion of bile acids, and some types of hyperlipoproteinemia may have associated abnormalities in biliary lipid metabolism (2–9). These observations suggested some form of coordination between the regulation of levels of cholesterol and cholesterol-derived metabolites in the bile and in the plasma. This coordination or linkage might be mediated through alterations in hepatocyte cholesterol metabolism, perhaps by regulatory processes necessary for maintenance of intracellular homeostasis.

Cholesterol homeostasis at the cellular level is maintained by a balance among influx, de novo synthesis, and efflux of cholesterol. In the liver, influx of cholesterol is largely through apoB-containing lipoproteins including those of intestinal origin that carry dietary components and that also contain apolipoprotein E. The lipoproteins are recognized, bound, and internalized by specific receptors on the cell surface (10–12). The liver also recognizes

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; VLDL, very low density lipoproteins; HDL, high density lipoproteins.

[1]A portion of this work was presented at the annual meeting of the American Heart Association in 1981 and was published in abstract form (1).

[2]Present address: Research Institute, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, CA 94301.

[3]To whom all correspondence should be addressed at: Department of Medicine, University of California, San Francisco and Metabolism Section, 111 F, VA Medical Center, 4150 Clement Street, San Francisco, CA 94121.

high density lipoproteins (HDL) (13, 14) that do not contain apoB or apoE, but that nevertheless can also deliver cholesterol to the liver (15, 16). The mechanism(s) of lipid delivery by HDL is less well understood.

After internalization and hydrolysis, the lipoprotein components are available to the cell for further use. The free fatty acids may be esterified and secreted as triglycerides, mainly in very low density lipoproteins (VLDL) (17). The free cholesterol has a number of potential fates. It can serve as a substrate for membrane and lipoprotein syntheses, for bile acid synthesis, for secretion into bile, and for esterification. What regulates the direction of flux of cholesterol into and out of these pathways is not completely understood.

The balance between cellular free and esterified cholesterol is thought to be determined largely by the activity of the enzyme acyl coenzyme A:cholesterol acyltransferase (ACAT) which is responsible for intracellular cholesterol esterification. Hepatic ACAT activity responds to perturbations known to alter hepatic cholesterol, bile acid, and lipoprotein metabolism (18–20). In addition, it has been shown that acute changes in the activity of this enzyme in vivo correlate with changes in biliary cholesterol content (21). In isolated rat hepatocytes, increases in ACAT activity are associated with increases in VLDL cholesteryl ester content (22). Thus, ACAT activity in the liver appears to be tightly regulated and coordinated with cellular requirements for free cholesterol. On the basis of the available data, it appears that ACAT is a regulatory point for maintenance of cholesterol homeostasis, and that it may play a role in controlling the flux of free cholesterol into different pathways within the hepatocyte.

In addition to cholesterol, chylomicrons and very low density lipoproteins contain large amounts of fatty acids, mainly as triglycerides. Thus, a fatty acid and cholesterol load is simultaneously delivered to the liver following uptake of these lipoproteins. This would be expected to alter hepatic cholesterol homeostasis in a fashion that reflects the overall lipid content of the particle. For example, uptake of a particle with a high cholesterol:triglyceride ratio could result acutely in an increase in cholesterol in the cell with little change in triglyceride secretion, while uptake of a particle with a low cholesterol:triglyceride ratio could result acutely in a lower cholesterol level within the cell and an increased VLDL triglyceride secretion induced by the fatty acid load. Under these circumstances, ACAT activity would be expected to respond differentially to reflect these opposing challenges to cholesterol homeostasis.

To test this hypothesis, lipoproteins containing different proportions of cholesterol and triglycerides were prepared, and their effects on ACAT activity were investigated both in vivo and in the isolated perfused liver. In addition, the rates of secretion of cholesterol and triglycerides in VLDL and of cholesterol in the bile in response to the lipoprotein infusions were studied in the perfused liver system.

MATERIALS AND METHODS

Chemicals

[1-^{14}C]Oleoyl coenzyme A (40–60 mCi/mmol) was obtained from New England Nuclear (Boston, MA) and [G-^{3}H]cholesterol (10 Ci/mmol) was from Schwarz-Mann. Cholesterol, cholesteryl oleate, oleic anhydride, oleic acid, triolein, oleoyl coenzyme A, coenzyme A, NADH, dimyristoyl phosphatidylcholine, 5,5'-dithiobis (2-nitrobenzoic acid), taurocholic acid, sodium taurocholate, Tween 80, propylthiouracil, and bovine serum albumin were from Sigma. Cholesterol oxidase (a kit for free cholesterol determination) was from Boehringer-Mannheim (Indianapolis, IN). Silica gel H was from E. Merck (Darmstadt, West Germany). Liquifluor was from New England Nuclear (Boston, MA). All other chemicals were reagent grade.

Animals

Male Sprague-Dawley rats were used in all experiments. They were housed for at least 7 days prior to use in a windowless room illuminated between 7:00 AM and 7:00 PM and fed a commercial rat chow ad libitum. Liver donors weighed 120–160 g; lymph donors weighed 300–400 g, and rats used in in vivo experiments weighed 180–220 g. Retired breeders were used for lipoprotein remnant preparation (23) and as plasma donors for cholesterol-rich, very low density lipoproteins after they had consumed an atherogenic diet (24) for 3 to 4 weeks.

Preparation of lipoproteins

Lipoproteins were prepared essentially as described previously (25). A silastic catheter was inserted into the mesenteric lymph duct of a 300–400 g rat (26). Twenty-four hours later standard chylomicrons were prepared by continuous infusion of a solution of one whole egg dispersed in 125 ml of normal saline through an intragastric catheter at a rate of 1.8 ml/hr. Chylomicrons enriched in triglycerides were prepared by a slight modification of the method of Bennett-Clark (27). A sonicated emulsion containing 7 g of triolein, 4 g of alpha-*d*-phosphatidylcholine, 0.1 g of sodium taurocholate, and 0.5 g of Tween 80 in 100 ml of H_2O was infused intragastrically into a lymph duct-cannulated rat, as described above. The lymph was collected in flasks containing 0.1 mg of gentamycin and 0.4 ml of 1% EDTA. The lymph was filtered through ten layers of gauze, layered under 0.9% NaCl, and centrifuged in a Beckman SW 41 rotor at 1×10^5 *g* for 45 min. The floating layer was separated and resuspended in a small volume of buffered normal saline.

Very low density lipoproteins enriched in cholesterol were prepared from a group of 20–30 retired breeders fed a diet containing 5% lard, 1% cholesterol, 0.1% propylthiouracil, and 3% taurocholic acid for a period of at least 3 weeks. The rats were killed between 1 PM and 3 PM by

aortic puncture and the serum was separated from red blood cells by low speed centrifugation. The VLDL was collected by adjusting the serum density to 1.019 g/ml with KBr and centrifuging at 1×10^5 *g* for 16 hr in a Beckman SW 41 rotor.

Preparation of lipoprotein remnants

Lipoprotein remnants were prepared based on the method of Redgrave (23). Eviscerated rats were prepared and placed in restraining cages. Native (precursor) lipoproteins, prepared as above, were injected at a rate of 0.6 ml/min through a femoral vein catheter. The injected lipoproteins contained no more than 8 mg of cholesterol in chylomicrons or cholesterol-rich VLDL or 2 mg of cholesterol in triglyceride-rich chylomicrons. Chylomicrons and triglyceride-rich chylomicrons were allowed to circulate for 3 hr. Cholesterol-rich VLDL was allowed to circulate for 30 min. The animals were exanguinated by aortic puncture and the blood was allowed to clot for 2 hr. Serum was separated from red blood cells by low speed centrifugation. Serum containing chylomicron remnants was centrifuged at 1×10^5 *g* for 2 hr in a Beckman SW 41 rotor. Serum containing VLDL remnants was adjusted to density 1.019 gm/ml and centrifuged at 1×10^5 *g* for 16 hr.

The relative proportions of triglyceride, cholesterol, phospholipid, and protein in these different lipoproteins were similar to those described previously (25).

In vivo administration of lipoproteins

Rats were lightly anesthetized with ether and a bolus of lipoproteins (7 mg of cholesterol/200 g body weight) was administered through a femoral vein catheter at a rate of 0.6 ml/min. The boli were delivered within 2–3 min at the most. At the end of 2 hr, the animals were killed and their livers were removed and placed in 0.9% NaCl on ice. Fractions were taken for the preparation of microsomes and assay of ACAT, cholesterol, or fatty acyl CoA as described below.

In some cases, samples were also taken for the preparation of microsomes and assay of HMG-CoA reductase (see ref. 25 for results).

Liver perfusion

After pentobarbital anesthesia, liver perfusion was performed in situ by the method of Mortimore (28). Blood flow was interrupted at most for a few seconds during preparation of the organ. Oxygenation was accomplished with a silastic coil (Dow Corning Corp. Midland, MI), as described by Hamilton et al. (29). The entire perfusion system, except the artificial lung, was siliconized. The plasma-free perfusate (40 ml, pH 7.4) consisted of 22% washed human red blood cells in Eagle's basal medium supplemented with 3 g of bovine serum albumin and 100 mg of glucose for each 100 ml of medium. Hemolysis during the perfusion was negligible. The perfusate circulated at 1.1 ml per g of liver/min. Oxygenation prior to and during perfusion was maintained using a gas mixture containing 95% O_2 and 5% CO_2. Viability of the liver was judged at 5–10 min by color, O_2 extraction, and the absence of perfusate loss. Viability was maintained over the 2.5-hr perfusion period as judged by these criteria.

In the experimental perfusions to which the various lipoprotein remnants were added, after 20 min of perfusion with lipoprotein-containing perfusate, an aliquot of the lipoprotein-containing perfusate was taken for triglyceride determination as described below to estimate the amounts of lipoproteins removed. The liver was then washed out with approximately 60–80 ml of lipoprotein-free perfusate to remove residual lipoproteins. The final washout perfusate contained no detectable triglycerides, indicating the effectiveness of the washout. This washout was shown to be effective in removing residual ^{125}I-labeled remnants from the perfused liver (L. Brewer, E. Daniels, and A. Cooper, unpublished observations). Recirculation with lipoprotein-free perfusate was reestablished, and perfusion was continued for an additional 2 hr. Hourly samples were taken for triglyceride determination. At the end of the perfusion, the final perfusate was collected, its hematocrit and volume were recorded, and the red cells were removed by low speed centrifugation. The liver was removed, weighed, chilled in 0.9% NaCl, and a portion was taken for preparation of microsomes. A sample of the perfusate was taken for triglyceride determination and the remainder was used for isolation of VLDL by ultracentrifugation as described above.

In one group of experiments, instead of lipoproteins, oleic acid complexed to albumin (17) was added to the perfusate at the concentration of 1 mM. Oleic acid concentration was maintained constant over the entire perfusion period. Free fatty acid uptake was equated with the amount infused (10 mg/g liver per 2 hr). The remainder of the perfusion protocol was as described above.

Collection of bile and analysis of biliary cholesterol

Livers were prepared for perfusion as described above and the bile duct was cannulated. Bile flow was 40–50 μl/g of liver per hr over the course of the experiment. Biliary cholesterol was determined on portions of bile that had been collected during the last 2 hr of perfusion. Portions were saponified and extracted and the cholesterol was determined either by the $FeCl_3$ method as described previously (30) or by the cholesterol oxidase method (Boehringer-Mannheim).

Preparation of microsomes

The liver samples, either from perfused livers or obtained from livers of animals in the in vivo experiments were blotted dry, weighed, minced, and homogenized in five volumes of buffer A (0.1 M sucrose, 0.05 M KCl, 0.02 M

KH_2PO_4, 0.03 M EDTA, pH 7.4) by three strokes at moderate speed in a glass-Teflon Potter-Elvehjem homogenizer.

The homogenate was centrifuged for 15 min at 10,000 *g* to sediment unbroken cells, nuclei, mitochondria, and lysosomes. The pellet was discarded and the microsomes were collected by centrifugation of the supernatant at 1×10^5 *g* for 60 min. The microsomal pellet was washed by suspension in buffer A and centrifugation at 1×10^5 *g* for 60 min. The final pellet was resuspended in buffer B containing 0.25 M sucrose, 0.001 M EDTA, and 0.1 M Tris-HCl, pH 7.5.

Assay of ACAT activity

ACAT activity was assayed essentially as described previously (18). The assay contained 0.04–0.2 mg of microsomal protein in buffer B at a final volume of 0.2 ml. The mixture was incubated for 6 min at 37°C with shaking, and the reaction was started by addition of 5 nmol of [^{14}C]oleoyl coenzyme A (specific activity, 20,000 dpm/nmol). The assay was stopped after 4 min by addition of 2 ml of chloroform–methanol 2:1. [^{3}H]Cholesteryl oleate (10,000 cpm), prepared as described previously (18), was added as internal standard to estimate recovery, followed by an additional 3 ml of chloroform–methanol followed by 1 ml of acidic H_2O. The samples were allowed to stand at 4°C overnight, the aqueous phase was removed, and the remainder was taken to dryness under N_2. The residue was dissolved in chloroform and plated on silica gel H. Cholesteryl oleate or linoleate and triolein were plated as markers. The plates were developed in hexane–ethyl acetate 9:1 (v/v), dried and visualized with I_2 vapor. The bands corresponding to cholesteryl esters were scraped into scintillation vials and toluene-Liquifluor was added. The samples were counted in a Beckman liquid scintillation counter. All values were corrected for quenching and channel spillover.

For each lot of substrate, a substrate blank was determined. [^{3}H]Cholesteryl oleate used as internal recovery standard was assayed by thin-layer chromatography to monitor its radiochemical purity in each experiment.

Assay of microsomal fatty acyl coenzyme A content

Fatty acyl coenzyme A content was estimated fluorimetrically, essentially as described by Garland, Shepherd, and Yates (31), using ketoglutaric oxidase (EC 1.2.4.2) prepared from fresh pig heart as described by Sanadi, Littlefish, and Bock (32). Rat liver microsomes (200 μl containing 3–4 mg of protein) were incubated for 30 min at 37°C with equal volumes of 0.85 M KOH and 1 M Tris base, pH 12. The mixture was then centrifuged to remove denatured protein. The assay contained, in a quartz cuvette, 100 nmol of potassium arsenate, pH 7.0, 2 nmol of EDTA, 4 nmol of L-cysteine, 2 nmol of potassium oxoglutarate, 0.3 nmol of NAD, and supernatant in a final volume of 2.0 ml in phosphate buffer, pH 7.4. The cuvette was placed in a fluorimeter (Perkin-Elmer model MPF 3) with excitation set at 345 nm and emission at 455 nm, and the initial reading was recorded. The reaction was started by addition of 2 μl of ketoglutaric oxidase, and followed for 40 min (end point value). Reference curves were constructed for both NADH and for coenzyme A. The limit of detectability was 0.1–0.2 nmol of coenzyme A.

Other assays

Protein was estimated according to Lowry et al. (33) or by the biuret method (34) using bovine serum albumin as reference standard. Phospholipid phosphorus was determined according to Bartlett (35) after total lipid extraction as described previously (30). Triglycerides were assayed enzymatically by the method of Eggstein and Kreutz (36) using the triglyceride assay kit from Boehringer-Mannheim. Unless otherwise stated, total cholesterol was determined by gas–liquid chromatography based on the method of Ishikawa et al. (37) as described previously (30).

RESULTS

Effect of lipoprotein composition on hepatic ACAT activity in vivo

To test the response of hepatic ACAT to the simultaneous influx of different amounts of cholesterol and triglyceride, lipoproteins of different lipid compositions were prepared. The triglyceride:cholesterol ratios by weight averaged 136 for the triglyceride-rich chylomicrons, 34 for the standard chylomicrons, and 0.47 for the cholesterol-rich VLDL (**Table 1**). Lipoproteins were administered intravenously as a bolus containing 7 mg of cholesterol/200 g body weight and the animals were killed 2 hr later. This concentration was chosen because, in preliminary studies, we found that in vivo administration of this amount of cholesterol as cholesterol-rich VLDL increased ACAT activity within 2 hr.

Administration of the triglyceride-rich chylomicrons resulted in 80% inhibition of microsomal ACAT activity (**Table 2**) while the cholesterol-rich VLDL induced a 44% increase in activity (Table 2). A bolus of the standard chylomicrons of intermediate composition showed 24% suppression which was statistically significant at $P < 0.01$ compared to control (Table 2). Thus, in vivo, ACAT activity appeared to respond not only to the lipoprotein cholesterol load but also to the triglyceride.

Effect of lipoprotein remnant composition on ACAT activity in the perfused liver

The perfused liver system was used to study further the effects of lipoprotein lipid composition on ACAT and on lipoprotein and biliary cholesterol metabolism.

Remnants prepared from the lipoproteins described

TABLE 1. Cholesterol and triglyceride ratios of chylomicrons and VLDL and their remnants and triglyceride and cholesterol removal from the perfusate by the isolated perfused rat liver

Lipoproteins	Triglyceride:Cholesterol		Triglyceride Removed	Cholesterol Removed
	Native	Remnant		
	wt/wt		*mg/g liver*	
Standard chylomicrons	33.9 ± 1.2 (4 prep.)	11.4 ± 1.6 (7 prep.)	2.4 ± 0.40 (7 detn.)	0.23 ± 0.02 (7 detn.)
Triglyceride-rich chylomicrons	136.5 ± 41.0 (4 prep.)	32.7 ± 10.6 (5 prep.)	2.81 ± 0.29 (7 detn.)	0.14 ± 0.03 (7 detn.)
Cholesterol-rich VLDL	0.47 ± 0.15 (3 prep.)	0.17 ± 0.01 (4 prep.)	0.30 ± 0.08 (6 detn.)	1.83 ± 0.60 (6 detn.)

Lipoproteins and their remnants were prepared as described in Methods. Livers were prepared as described in Methods. A bolus of lipoproteins was added to the perfusate and the perfusate was allowed to recirculate for 20 min, after which the perfusate was collected and analyzed for the triglyceride content. The amounts of cholesterol removed were calculated from the amounts of triglyceride removed, assuming that the triglyceride:cholesterol ratio for each lipoprotein preparation reflected that of the lipoproteins removed from the perfusate. All values are ± SEM. The numbers of determinations are in parentheses.

above had average triglyceride:cholesterol weight ratios of 32.7 for the triglyceride-rich chylomicron remnants, 11.4 for standard chylomicron remnants, and 0.17 for cholesterol-rich VLDL remnants (Table 1).

ACAT activity in the control perfused livers was increased relative to what would have been expected for such livers in vivo (**Table 3**). The mechanism of this increase is unknown, but it may reflect absence of hormonal or other factors normally present in the circulation, or an acute effect of removing the enterohepatic circulation which might lead to changes in cholesterol availability to the enzyme.

Only inclusion of the triglyceride-rich chylomicron remnants affected ACAT activity significantly relative to the control, lipid-free perfusate. ACAT activity was decreased 54% (Table 3). Standard chylomicrons and cholesterol-rich VLDL remnants had little effect.

The amounts of lipoprotein triglyceride removed during the 20-min perfusions with the standard chylomicron remnants and triglyceride-rich chylomicrons were not significantly different (Table 1). In the triglyceride-rich chylomicron remnant perfusions, 3.5 ± 0.6 mg of cholesterol (average of nine determinations) was added to the perfusate, while 4.2 ± 0.4 mg of cholesterol (average of six determinations) as standard chylomicron remnants was added. In both cases, 30–40% of the total bolus was removed in the 20-min perfusion time. The amounts of hypercholesterolemic VLDL remnants added to the perfusates varied over a wider range; again, about 30–40% of the lipoprotein cholesterol added was removed over the 20-min time period.

Because ACAT activity both in vivo and in the perfused liver system was decreased by administration of triglyceride-rich lipoproteins, the effect of free fatty acid administration on ACAT was investigated in the perfused liver system. Infusion of oleic acid complexed to albumin decreased ACAT activity to levels similar to those obtained with infusion of the triglyceride-rich chylomicron remnants (0.11 ± 0.02 nmol of cholesteryl oleate min^{-1} mg of $protein^{-1}$; average of 16 determinations).

TABLE 2. Effect in vivo of native (precursor) lipoproteins on hepatic ACAT activity and microsomal free cholesterol content

Treatment	ACAT Activity	Free Cholesterol
	nmol CE/min per mg protein	*μg/mg protein*
Control (18)	0.153 ± 0.012	26.3 ± 0.7
Chylomicrons (9)	0.116 ± 0.003[b]	30.1 ± 1.6
Triglyceride-rich chylomicrons (8)	0.031 ± 0.005[c]	20.7 ± 1.4[a]
Cholesterol-rich VLDL (9)	0.221 ± 0.009[c]	33.2 ± 2.0[a]

Lipoproteins containing 7 mg of cholesterol and different amounts of triglycerides were injected into rats through a femoral vein catheter. The animals were killed 2 hr later, the livers were excised, microsomes were prepared, and ACAT activity and cholesterol content were determined. The numbers in parentheses are the numbers of animals. Values are ± SEM.

[a] Statistically significantly different from control at $P < 0.05$ by Student's unpaired two-tailed t-test.
[b] Different at $P < 0.01$.
[c] Different at $P < 0.001$.

TABLE 3. Effect of lipoprotein remnant composition on parameters of cholesterol metabolism in the perfused liver

Perfusion	ACAT Activity	Microsomal Total Cholesterol	Secreted Total Cholesterol		Secreted Triglyceride VLDL
			VLDL	Bile	
	nmol CE/min per mg protein	*μg/mg protein*	*μg/hr per g liver*		
Control (6)	0.39 ± 0.05	30 ± 3	43 ± 6	19 ± 4	338 ± 21
Chylomicron remnants (6)	0.35 ± 0.05	33 ± 3	68 ± 9[a]	20 ± 5	397 ± 22[c]
Triglyceride-rich chylomicron remnants (9)	0.18 ± 0.06[d]	19 ± 2[b]	72 ± 6[c]	13 ± 2[e]	632 ± 55[d]
Cholesterol-rich VLDL remnants (5)	0.30 ± 0.03	30 ± 2	52 ± 15	16 ± 2	287 ± 71

Livers were perfused as described in Methods. Viability of perfusion was established and lipoprotein remnants were added and allowed to circulate for 20 min. Lipoproteins remaining were washed out and perfusion was continued for an additional 2 hr, during which bile was also collected. The livers were removed, microsomes were prepared and assayed for ACAT activity and cholesterol content. The perfusate was collected and VLDL was isolated and assayed for cholesterol and triglyceride contents. The bile was assayed for cholesterol content. The numbers in parentheses are the numbers of animals. All values are mean ± SEM.

[a] Statistically significantly different from control at $P < 0.05$ by Student's unpaired two-tailed *t*-test.
[b] Different from control at $P < 0.02$.
[c] Different from control at $P < 0.01$.
[d] Different from control at $P < 0.005$.
[e] Different from control at $P < 0.05$ (one-tailed *t*-test).

Relationship of ACAT activity to cholesterol load and to microsomal free cholesterol content

To investigate why ACAT activity responded differently to the various lipoproteins despite administration of similar amounts of cholesterol, possible regulatory factors were studied. There was no correlation of ACAT activity in vivo or in the perfused liver system with cholesterol load. In vivo a constant bolus of cholesterol was administered and ACAT activities ranged from 30 pmol of cholesteryl oleate min^{-1} mg of $protein^{-1}$ to 221 pmol min^{-1} mg of $protein^{-1}$ (Table 2). In the perfused liver, removal of cholesterol from the perfusate ranged from 0.05 to 4.7 mg of cholesterol g of $liver^{-1}$ with an average value of 0.23 mg for standard chylomicron remnants, 0.14 mg for triglyceride-rich remnants, and 1.83 mg for the cholesterol-rich VLDL remnants (Table 1). There was little correlation of ACAT activity with the amounts of cholesterol removed. This suggested that ACAT activity was not regulated solely by the amount of cholesterol delivered to the hepatocyte.

In vivo, the microsomal free cholesterol content increased with administration of hypercholesterolemic VLDL correlating with the increase in ACAT activity. In contrast, after administration of the triglyceride-rich chylomicrons, the microsomal free cholesterol content decreased in parallel with the decrease in ACAT activity (Table 1). In the perfused liver system only liver microsomes from the perfusions with triglyceride-rich chylomicron remnants showed a statistically significant change in the cholesterol content (Table 3). This decrease correlated with the decrease in ACAT activity.

Thus, although there was little relationship of ACAT activity to amounts of lipoprotein cholesterol administered or removed, there were correlations with microsomal cholesterol content.

Relationship of ACAT activity to microsomal fatty acyl CoA levels

In some cases large amounts of lipoprotein triglycerides were infused; thus, it was possible that increases in microsomal fatty acyl CoA concentrations were responsible for the observed decreases in ACAT activity assayed in vitro. There were no changes in the microsomal fatty acyl CoA content within the limits of detectability after any treatment, either in vivo or in the perfused liver. All values were approximately 0.2 nmol mg of $protein^{-1}$, in good agreement with the values reported by Garland et al. (31) for liver microsomes from untreated rats. Thus changes in microsomal fatty acyl CoA pool size are not likely to be responsible for the differences observed in ACAT activity assayed in vitro. However, it is possible that, in the intact cell, the regulation of cytosolic fatty acyl CoA levels and their direction towards phospholipid, triglyceride, or cholesteryl ester synthesis or fatty acyl CoA hydrolysis, as proposed by Ockner et al. (38) and by Pikkukangas et al. (39), may play a role in regulating cholesteryl ester synthesis.

Regulation of ACAT activity by phosphorylation

It has been suggested that ACAT activity can be regulated by a phosphorylation–dephosphorylation mechanism (40, 41), with phosphorylation associated with increased activity. Administration of cholesterol-rich VLDL in vivo acutely increased ACAT activity, suggesting that this mechanism might have been responsible. Preparation of liver microsomes in the presence of NaF, a phosphatase inhibitor, results in a preparation in which the phosphorylation state of the proteins may more closely reflect the in vivo situation (42). ACAT activity assayed in microsomes from control animals prepared in the presence of 50 mM NaF was not significantly different from activity

assayed in microsomes prepared in the absence of NaF (0.16 ± 0.02 nmol of cholesteryl oleate min^{-1} mg of $protein^{-1}$ vs. 0.17 ± 0.02, average of seven animals). In contrast, ACAT activity in microsomes prepared from animals administered cholesterol-rich VLDL in vivo did show differences: in microsomes prepared in the absence of NaF, the average value was 0.20 ± 0.02 nmol of cholesteryl oleate min^{-1} mg of $protein^{-1}$, while in those prepared in the presence of NaF, it was 0.26 ± 0.03 ($P < 0.01$ by paired t-test). This suggested that the increase in hepatic ACAT activity triggered by cholesterol-rich lipoprotein administration may have been mediated in part by phosphorylation of the enzyme itself or of a factor(s) associated with it. There were no differences observed in ACAT activity in microsomes, prepared in the presence and absence of NaF, from animals administered triglyceride-rich lipoproteins (data not shown).

Interrelationships of other parameters of hepatic cholesterol metabolism

In the rat, a portion of the cholesteryl esters in VLDL are of hepatic origin (43, 44). Further, studies with isolated hepatocytes (22) suggested that there are correlations between the levels of ACAT activity and the amounts of cholesteryl ester secreted in VLDL. Other studies (21, 45) suggest a relationship between ACAT activity and biliary cholesterol levels. Using the perfused liver system we investigated possible interrelationships of the amounts of lipoprotein cholesterol and triglyceride removed with the amounts of cholesterol secreted in VLDL and in the bile.

In control lipoprotein-free perfusions, there was an inverse linear correlation between VLDL triglyceride and cholesterol secretion and biliary cholesterol secretion (**Fig. 1A and B,** $r = 0.81$), suggesting that the cholesterol for both these processes was derived in part from the same or interrelated pools.

Biliary cholesterol content declined with increasing triglyceride uptake (**Fig. 2,** $r = 0.74$). Together with the data above, this suggested acute diversion of cholesterol to VLDL synthesis. ACAT activity also decreased under these circumstances as did microsomal cholesterol content (Table 3).

There were correlations of microsomal cholesterol content with biliary cholesterol secretion (**Fig. 3A,** $r = 0.58$) and with VLDL triglyceride secretion (Fig. 3B, $r = 0.43$), suggesting precursor pools were to be found among these cellular membrane elements. Correlations between ACAT activity and VLDL or biliary cholesterol secretion were complex in this system.

DISCUSSION

Cellular cholesterol homeostasis is maintained by a balance among influx and efflux of cholesterol, its rate of de novo synthesis, and its storage as cholesteryl esters. In the liver, influx of cholesterol is principally via apoB-containing lipoproteins including those of intestinal origin that carry dietary-derived lipids and also contain apoE. The main routes of efflux of cholesterol from the hepatocyte are as bile acids, as biliary cholesterol, or as lipoprotein cholesterol, both free and esterified.

From the studies reported here it is clear that both the cholesterol and triglyceride components of influxing lipoproteins can play a role in determining the response of the hepatocyte to lipoproteins. In the present study, the rates of VLDL triglyceride and cholesterol secretion were both increased by a bolus of triglyceride-rich chylomicron remnants, confirming that acute delivery of a fatty acid load as lipoprotein triglyceride can induce hepatic VLDL

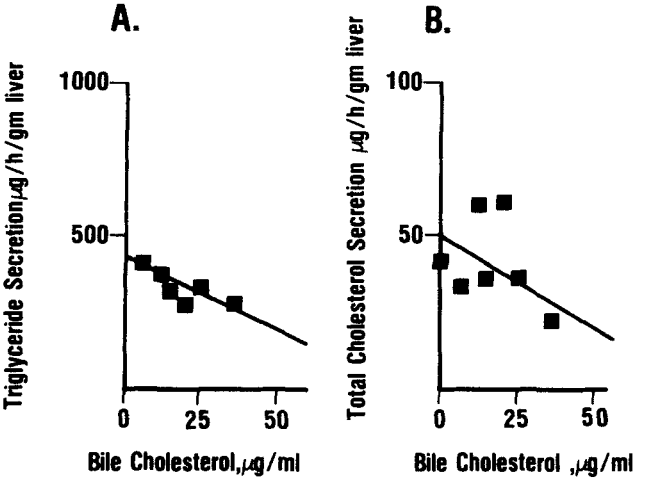

Fig. 1. Relationship of VLDL triglyceride (A) and VLDL cholesterol (B) secretion to biliary cholesterol secretion. Livers were perfused. Bile was collected over the 2-hr period and assayed for cholesterol content. At the end of 2 hr, the perfusate was collected and VLDL was isolated by centrifugation and assayed for triglyceride and cholesterol contents. Each point represents the values from one animal.

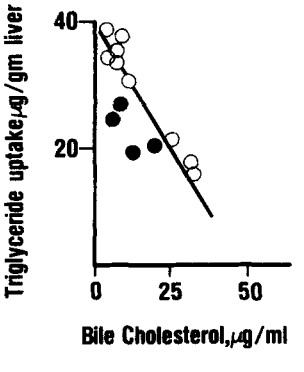

Fig. 2. Relationship of biliary cholesterol secretion to hepatic triglyceride uptake. Livers were perfused and bile was collected. Triglyceride uptake was measured as the difference in the amounts of triglyceride in the perfusate at t = 0 and the amount remaining at t = 30 min; (○) standard chylomicron remnants; (●) triglyceride-rich chylomicron remnants.

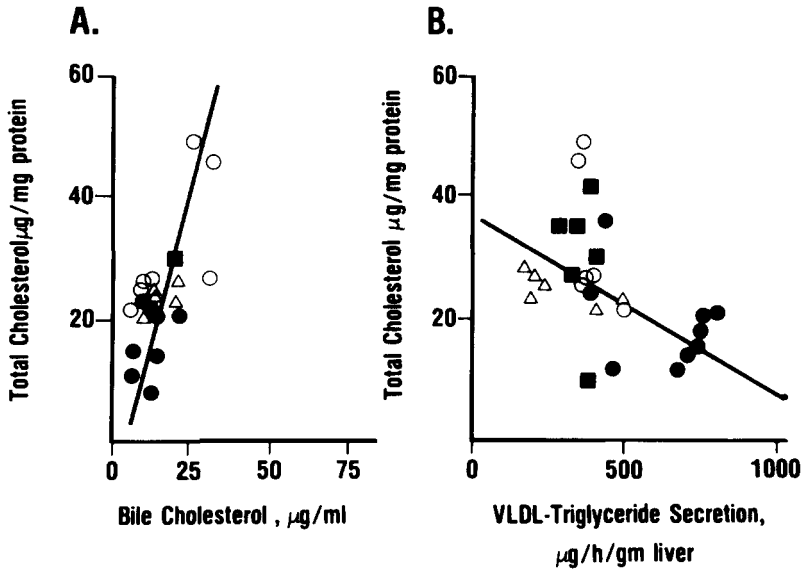

Fig. 3. Relationship of liver microsomal cholesterol content to biliary cholesterol secretion (A) and to VLDL triglyceride secretion (B). Livers were perfused and bile was collected. At the end of 2 hr, perfusate VLDL was isolated by centrifugation and assayed for triglycerides. The bile was analyzed for cholesterol. The livers were removed and a microsomal fraction was prepared and assayed for cholesterol content. Each point represents values from one animal; (○) control; (●) triglyceride-rich chylomicron remnant preperfusion; (■) standard chylomicron remnant preperfusion; (△) hypercholesterolemic VLDL remnant preperfusion.

secretion (25). In addition, it was found that, concomitant with the increased secretion of cholesterol in VLDL, the amount of cholesterol excreted into the bile after administration of these lipoproteins was decreased. This suggested the possibility that the biliary cholesterol precursor pool may have been diverted, at least partially, towards lipoprotein production. Moreover, hepatic ACAT activity was decreased both by perfusion of triglyceride-rich chylomicron remnants and by infusion of oleic acid complexed to albumin. The effects of oleic acid infusion, which is known to increase VLDL secretion (17), on biliary cholesterol content were not measured in this study.

In previous work we reported that acute alteration of ACAT activity affected the rate of biliary cholesterol secretion (21). The observations in this report further strengthen the hypothesis that there is a common pool of cholesterol that is a source for biliary cholesterol secretion and a substrate for ACAT, and they suggest that this common pool is also shared with the precursor pool for VLDL cholesterol secretion.

There was a modest but significant decrease in microsomal cholesterol content concomitant with the decrease in ACAT activity and in biliary cholesterol secretion, and with the increase in VLDL cholesterol accumulation induced by the triglyceride-rich remnants. This is consistent with the suggestion that the biliary cholesterol precursor pool is contained within this subcellular fraction (46–48) as is the cholesterol substrate pool for ACAT and for VLDL synthesis. Moreover, these results suggest that when the amounts of cholesterol delivered in lipoproteins together with that generated by in situ synthesis are insufficient to compensate for increases in lipoprotein secretion induced by the triglyceride load, cholesterol can be diverted from biliary secretion in an effort to maintain cellular homeostasis. Thus, the biliary and lipoprotein cholesterol precursor pools must be either common or in rapid equilibrium with one another.

The observation that standard chylomicron remnants of intermediate triglyceride content had only a modest effect on ACAT activity and did not cause statistically significant changes in microsomal cholesterol content or in the amount of cholesterol secreted into the bile suggests that influx of these particles did not acutely disturb the hepatic cholesterol homeostatic mechanism enough to invoke compensatory changes in ACAT or biliary cholesterol content despite the increase in VLDL cholesterol secretion. In previous work from this laboratory (25), similar lipoprotein remnants had little effect on HMG-CoA reductase activity. Thus, taken together, these results suggest that, in the case of these lipoproteins, the amounts of cholesterol that entered the cell together with the triglyceride load were sufficient to balance efflux of fatty acid equivalents and cholesterol in VLDL without acute compensation by other factors responsible for maintenance of cellular cholesterol homeostasis.

Although administration of cholesterol-rich VLDL in vivo did result in increased ACAT activity, administration of remnants of these lipoproteins as a bolus to the perfused liver had little effect. There were also no statistically significant effects on cholesterol secretion in VLDL or in

the bile. We reported previously that perfusion of similar lipoproteins suppressed HMG-CoA reductase activity (25). Taken together, the data suggest that under these conditions suppression of synthesis was sufficient to maintain homeostasis. This supports the suggestion of Spady, Turley, and Dietschy (49) that the initial response to alterations of hepatic cholesterol homeostasis is to change the cholesterol synthetic rate. This is also in agreement with the finding of Turley and Dietschy (50) that chylomicron lipoprotein cholesterol uptake is dissociated from biliary cholesterol content.

On the basis of the above, it appears that hepatic ACAT activity is responsive to both the cholesterol and triglyceride contents of lipoproteins taken up by the liver, and the results suggest that the response of ACAT is coordinated with other parameters important in maintaining cholesterol homeostasis in the hepatocyte. Whether the response of ACAT reflects a primary effect on the enzyme itself or secondary effects due to other perturbations induced by these lipoproteins is difficult to assess at present. The detailed mechanisms of regulation of ACAT activity have not yet been elucidated.

Based on these and other data, the following model appears reasonable. Cholesterol that is newly synthesized in the endoplasmic reticulum can serve preferentially as a substrate for bile acid synthesis, cholesterol esterification, and VLDL assembly, in part because these processes are all localized to the same subcellular organelle. A portion of the cholesterol destined for biliary secretion can also be derived from this newly synthesized sterol. Thus, changes in any one of these processes can rapidly affect the others. Free cholesterol liberated by lysosomal hydrolysis of removed serum lipoproteins or from turnover of cell membranes can be transported in part to the endoplasmic reticulum and can equilibrate with the newly synthesized sterol. As sterol accumulates, the rates of cholesterol synthesis and esterification compensate. As cholesterol continues to accumulate, the amounts of cholesterol secreted in lipoproteins may increase and bile acid synthesis and secretion and biliary cholesterol secretion may also increase in an effort to maintain intracellular cholesterol homeostasis. Lipoprotein receptors may also down-regulate in a further attempt to maintain homeostasis. When cholesterol influx is low or demands are increased, for example, by fatty acid or triglyceride influx, cholesterol synthesis increases, esterification decreases, and cholesterol may be diverted into one or another secretory pathway. If these demands continue, lipoprotein receptors may up-regulate in an effort to balance the increased efflux of cholesterol and its metabolites. The priorities determining availability of cholesterol to each pathway, what regulates these and how this is achieved, are at present unknown and will require further experimentation.

We want to thank Ms. M. Adeline Shrewsbury for assistance with some of the early experiments, Ms. Anna Iosiphidis for excellent technical assistance with the perfused liver studies, and Ms. Maggie Joe for typing the manuscript. Dr. Robert Simoni kindly provided us with the use of his fluorimeter. This work was supported by grants HL-05360 and AM-18774 from the National Institutes of Health. Dr. Van Zuiden was supported by NIH training grant #07056 while at Stanford University and by a grant (to P. E. A. V. Z., Gastroenterology Section, VA Medical Center, Providence, Rhode Island) from the Veterans Administration (Research Advisory Group).

Manuscript received 11 July 1986, in revised form 1 December 1986, and in re-revised form 9 March 1987.

REFERENCES

1. Van Zuiden, P., A. Cooper, and S. Erickson. 1981. Effects of lipoprotein composition on regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A:cholesterol acyltransferase. *Circulation.* **64:** 1027.
2. Angelin, B., K. Einarsson, K. Hellström, and B. Leijd. 1978. Bile acid kinetics in relation to endogenous triglyceride metabolism in various types of hyperlipoproteinemia. *J. Lipid Res.* **19:** 1004–1016.
3. Pertsemlidis, D., D. Panveliwalla, and E. H. Ahrens, Jr. 1974. Effects of clofibrate and of an estrogen-progestin combination on fasting biliary lipids and cholic acid kinetics in man. *Gastroenterology.* **66:** 565–573.
4. Grundy, S. M., E. H. Ahrens, Jr., G. Salen, P. H. Schreibman, and P. J. Nestel. 1972. Mechanisms of action of clofibrate on cholesterol metabolism in patients with hyperlipidemia. *J. Lipid Res.* **13:** 531–551.
5. Zorilla, E., M. Hulse, and A. Hernandez. 1968. Severe endogenous hypertriglyceridemia during treatment with estrogen and oral contraceptives. *J. Clin. Endocrinol. Metab.* **28:** 1793–1796.
6. Ahlberg, J., B. Angelin, I. Björkhem, K. Einarsson, and B. Leijd. 1979. Hepatic cholesterol metabolism in normo- and hyperlipidemic patients with cholesterol gallstones. *J. Lipid Res.* **20:** 107–115.
7. Albers, J. J., S. M. Grundy, P. A. Cleary, D. M. Small, J. M. Lachin, L. J. Schoenfield, and the National Cooperative Gallstone Study Group. 1981. National Cooperative Gallstone Study. The effect of chenodeoxycholic acid on lipoproteins and apolipoproteins. *Gastroenterology.* **82:** 638–646.
8. Angelin, B., K. Einarsson, K. Hellström, and B. Leijd. 1978. Effects of cholestyramine and chenodeoxycholic acid on the metabolism of endogenous triglyceride in hyperlipoproteinemia. *J. Lipid Res.* **19:** 1017–1024.
9. Portman, O. W., M. Alexander, N. Tanaka, and T. Osuga. 1980. Relationship between cholesterol gallstones, biliary function and plasma lipoproteins in squirrel monkeys. *J. Lab. Clin. Med.* **96:** 90–101.
10. Cooper, A. D., S. K. Erickson, R. Nutik, and A. Shrewsbury. 1982. Characterization of chylomicron remnant binding to rat liver membranes. *J. Lipid Res.* **23:** 42–52.
11. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1982. Lipoprotein binding to canine hepatic membranes. Metabolically distinct apoE and apoB/E receptors. *J. Biol. Chem.* **256:** 5646–5655.
12. Kita, T., J. L. Goldstein, M. S. Brown, Y. Watanabe, C. A. Hornick, and R. J. Havel. 1982. Hepatic uptake of chylomicron remnants in WHHL rabbits: a mechanism genetically distinct from the low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* **79:** 3623–3627.

13. Roheim, P. S., D. Rachmilewitz, O. Stein, and Y. Stein. 1971. Metabolism of iodinated high density lipoproteins in the rat. *Biochim. Biophys. Acta.* **248:** 315–329.
14. Hoeg, J. M., S. J. Demoskey, Jr., S. B. Edge, R. E. Gregg, J. C. Osborne, Jr., and H. B. Brewer, Jr. 1985. Characterization of a human hepatic receptor for high density lipoprotein. *Arteriosclerosis.* **5:** 228–237.
15. Sigurdsson, G., S. P. Noel, and R. J. Havel. 1979. Quantification of the hepatic contribution to the catabolism of high density lipoproteins on the rat. *J. Lipid Res.* **20:** 316–324.
16. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.
17. Heimberg, M., M. G. Wilcox, G. D. Dunn, W. F. Woodside, K. J. Breen, and G. Soler-Argilaga. 1974. Studies on the regulation of secretion of the very low density lipoprotein and on ketogenesis by the liver. *In* Regulation of Hepatic Metabolism. F. Lundquist and N. Tygstrup, editors. Munksgaard, Copenhagen. 110–143.
18. Erickson, S. K., M. A. Shrewsbury, C. Brooks, and D. J. Meyer. 1980. Rat liver acyl-coenzyme A:cholesterol acyltransferase: its regulation in vivo and some of its properties in vitro. *J. Lipid Res.* **21:** 930–941.
19. Balasubramaniam, S., K. A. Mitropoulos, and S. Venkatesan. 1978. Rat liver acyl-CoA:cholesterol acyltransferase. *Eur. J. Biochem.* **90:** 377–378.
20. Drevon, C. A., D. B. Weinstein, and D. Steinberg. 1980. Regulation of cholesterol esterification and biosynthesis in monolayer cultures of normal adult rat hepatocytes. *J. Biol. Chem.* **255:** 9128–9137.
21. Stone, B. G., S. K. Erickson, W. Y. Craig, and A. D. Cooper. 1985. Regulation of rat biliary cholesterol secretion by agents that alter intrahepatic cholesterol metabolism. *J. Clin. Invest.* **76:** 1773–1781.
22. Drevon, C. A., S. C. Engelhorn, and D. Steinberg. 1980. Secretion of very low density lipoproteins enriched in cholesteryl esters by cultured rat hepatocytes during stimulation of intracellular cholesterol esterification. *J. Lipid Res.* **21:** 1065–1071.
23. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49:** 465–471.
24. Mahley, R. W., and K. S. Holcombe. 1977. Alterations of the plasma lipoprotein and apoproteins following cholesterol feeding in the rat. *J. Lipid Res.* **18:** 314–324.
25. Van Zuiden, P. E. A., S. K. Erickson, and A. D. Cooper. 1983. Effect of removal of lipoproteins of different composition on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and hepatic very low density lipoprotein secretion. *J. Lipid Res.* **24:** 418–428.
26. Bollman, J. L., J. C. Cain, and J. H. Grindly. 1948. Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. *J. Lab. Clin. Med.* **33:** 1349–1352.
27. Bennett-Clark, S. 1978. Chylomicron composition during duodenal triglyceride and lecithin infusion. *Am. J. Physiol.* **235:** E183–190.
28. Mortimore, G. E. 1961. Effect of insulin on potassium transfer in isolated rat liver. *Am. J. Physiol.* **200:** 1315–1319.
29. Hamilton, R. L., M. N. Berry, M. C. Williams, and E. M. Sevringhaus. 1974. A simple and inexpensive membrane "lung" for small organ perfusion. *J. Lipid Res.* **15:** 182–186.
30. Erickson, S. K., A. D. Cooper, S. M. Matsui, and R. G. Gould. 1977. 7-Ketocholesterol: its effect on hepatic cholesterogenesis and its hepatic metabolism in vivo and in vitro. *J. Biol. Chem.* **252:** 5186–5193.
31. Garland, P. B., D. Shepherd, and D. W. Yates. 1965. Steady-state concentrations of coenzyme A, acetyl-coenzyme A and long-chain fatty acyl-coenzyme A in rat liver mitochondria oxidizing palmitate. *Biochem. J.* **97:** 587–594.
32. Sanadi, D. R., J. W. Littlefish, and R. M. Bock. 1952. Studies on alpha-keto-glutaric oxidase. II. Purification and properties. *J. Biol. Chem.* **197:** 851–861.
33. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
34. Gornall, A. G., C. J. Bardawill, and M. M. David. 1948. Determination of serum protein by means of the biuret reaction. *J. Biol. Chem.* **177:** 751–756.
35. Bartlett, G. R. 1959. Phosphorous assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
36. Eggstein, M., and F. H. Kreutz. 1966. Eine neue Bestimmung der Neutralfette in Blutserum and Gewebe. I. Mitteilung. Princip, Durchfukrung und Besprechung der Methode. *Klin. Wochenschr.* **44:** 262–267.
37. Ishikawa, T. T., J. MacGee, J. A. Morrison, and C. J. Glueck. 1974. Quantitative analysis of cholesterol in 5 to 20 μl of plasma. *J. Lipid Res.* **15:** 286–291.
38. Ockner, R. K., N. Lysenko, J. A. Manning, S. E. Monroe, and D. A. Burnett. 1980. Sex steroid stimulation of fatty acid utilization and fatty acid binding protein concentration in rat liver. *J. Clin. Invest.* **65:** 1013–1023.
39. Pikkukangas, A. H., R. A. Vaanen, M. J. Savolainen, and I. E. Hassinen. 1982. Precursor supply and hepatic enzyme activities as regulators of triacylglycerol synthesis in isolated hepatocytes and perfused liver. *Arch. Biochem. Biophys.* **217:** 216–225.
40. Gavey, K. L., D. I. Trujillo, and T. J. Scallen. 1983. Evidence for phosphorylation/dephosphorylation of rat liver acyl-CoA:cholesterol acyltransferase. *Proc. Natl. Acad. Sci. USA.* **80:** 2171–2174.
41. Suckling, K. E., E. F. Stange, and J. M. Dietschy. 1983. Dual modulation of hepatic and intestinal acyl-CoA:cholesterol acyltransferase activity by (de-)phosphorylation and substrate supply in vitro. *FEBS Lett.* **151:** 111–116.
42. Brown, M. S., J. L. Goldstein, and J. M. Dietschy. 1979. Active and inactive forms of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Biol. Chem.* **254:** 5144–5149.
43. Mahley, R. W., R. L. Hamilton, and V. S. LeQuire. 1968. Characterization of lipoprotein particles isolated from the Golgi apparatus of rat liver. *J. Lipid Res.* **10:** 433–439.
44. Swift, L. L., P. D. Soulé, and V. S. LeQuire. 1982. Hepatic Golgi lipoproteins: precursors to plasma lipoproteins in hypercholesterolemic rats. *J. Lipid Res.* **23:** 962–971.
45. Nervi, F. O., R. Del Pozo, C. F. Covarrubias, and B. O. Ronco. 1983. The effect of progesterone on the regulatory mechanisms of biliary cholesterol secretion in the rat. *Hepatology.* **3:** 360–367.
46. Robins, S. J., and H. Brunengraber. 1982. Origin of biliary cholesterol and lecithin in the rat: contribution of new synthesis and preformed hepatic stores. *J. Lipid Res.* **23:** 604–608.
47. Long, T. T., L. Jakoi, R. Stevens, and S. Quarfordt. 1978. The sources of rat biliary cholesterol and bile acid. *J. Lipid Res.* **19:** 872–878.
48. Gregory, D. H., Z. R. Vlahcevic, P. Schatzky, and L. Swell.

1975. Mechanism of secretion of biliary lipids. I. Role of bile cannalicular and microsomal membranes in the synthesis and transport of biliary lecithin and cholesterol. *J. Clin. Invest.* **55:** 105–114.

49. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J. Lipid Res.* **26:** 465–472.

50. Turley, S. D., and J. M. Dietschy. 1979. Regulation of biliary cholesterol output in the rat: dissociation from the rate of hepatic cholesterol synthesis, the size of the hepatic cholesteryl ester pool, and the hepatic uptake of chylomicron cholesterol. *J. Lipid Res.* **20:** 923–934.