papers and notes on methodology

Studies on acyl-coenzyme A: cholesterol acyltransferase activity in human liver microsomes

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Abstract The aim of the present study was to characterize the acyl-coenzyme A: cholesterol acyltransferase (ACAT) activity in human liver microsomes. Liver biopsies were obtained from patients undergoing elective cholecystectomy under highly standardized conditions. In 34 patients the enzyme activity of the microsomal fraction averaged 6.6 ± 0.7 (mean ± SEM) pmol · min^{-1} · mg protein^{-1} in the absence of exogenous cholesterol. Freezing of the liver biopsy in liquid nitrogen increased the enzyme activity five- to sixfold. Similarly, freezing of the microsomal fraction prepared from unfrozen liver tissue increased the enzyme activity about twofold. These results may help to explain previous disparate results reported in the literature. The enhanced ACAT activity obtained by freezing was at least partly explained by a transfer of unesterified cholesterol to the microsomal fraction and possibly also by making the substrate(s) more available to the enzyme. Preincubation of the microsomal fraction, prepared from unfrozen liver tissue, with unlabeled cholesterol increased the enzyme activity about fivefold. This finding indicates that hepatic ACAT in humans can also utilize exogenous cholesterol as substrate. Addition of cholesterol to frozen microsomes prepared from unfrozen liver tissue increased the ACAT activity two- to threefold, whereas addition of cholesterol to microsomes prepared from frozen liver tissue did not further increase the enzyme activity. No evidence supporting the concept that ACAT is activated-inactivated by phosphorylation-dephosphorylation could be obtained by assaying the enzyme under conditions similar to those during which the human HMG-CoA reductase is inactivated-activated. —**Einarsson, K., L. Benthin, S. Ewerth, G. Hellers, D. Ståhlberg, and B. Angelin.** Studies on acyl-coenzyme A: cholesterol acyltransferase activity in human liver microsomes. *J. Lipid Res.* 1989. **30:** 739–746.

Supplementary key words dephosphorylation • phosphorylation

The formation of cholesteryl esters is an important pathway in the overall regulation of cholesterol metabolism in the human liver (1). Thus, the balance between the availability of free and esterified cholesterol may influence the flux of steroid into lipoproteins, bile acids, and biliary cholesterol. Considering the great impact of disturbances of cholesterol metabolism in various diseases, e.g., cholesterol gallstone disease and atherosclerosis, it is important to thoroughly characterize cholesteryl ester formation in human liver. The intracellular esterification of cholesterol is catalyzed by the microsomal enzyme acyl-coenzyme A:cholesterol acyltransferase (ACAT; EC 2.3.1.26). The occurrence of ACAT activity in human liver was first demonstrated by Balasubramaniam et al. (2) using homogenates of frozen tissue. These authors observed that the human enzyme preferentially uses endogenous microsomal cholesterol as substrate, and that exogenously added cholesterol is not well utilized by the enzyme. This finding was later confirmed by Erickson and Cooper (3). In contrast, Smith et al. (4) recently reported that preincubation of human liver microsomes with exogenous cholesterol produced an increse in ACAT activity. Furthermore, the range of enzyme activity reported for human hepatic ACAT is very wide (2–4).

The aim of the present work was to characterize in some detail the properties of the human microsomal ACAT activity. Particularly, we studied the effects of tissue handling (freeze–thawing), substrate availability, and the possible regulation by phosphorylation–dephosphorylation, as has been suggested for the rat liver enzyme (5–9).

Abbreviations: ACAT, acyl-coenzyme A: cholesterol acyltransferase (EC 2.3.1.26); EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis (betaaminoethyl ether)- N, N, N', N'-tetraacetic acid; HMG, 3-hydroxy-3-methylglutaryl; PMSF, phenylmethane-sulfonyl fluoride.

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MATERIALS AND METHODS

Materials

[1 – ^{14}C]Oleoyl coenzyme A (sp act 57.8 mCi/mmol) and [1,2,6,7 – ^{3}H]cholesteryl oleate (sp act 82.7 Ci/mmol) were obtained from New England Nuclear Corp., Boston, MA. Cholesteryl oleate, cholesterol, human serum albumin (free of fatty acids), Triton WR-1339, EDTA, EGTA, PMSF, and leupeptin were purchased from Sigma Chemical Co., St. Louis, MO. *E. coli* alkaline phosphatase, suspended in 2.6 M ammonium sulfate (30–60 units/mg protein) was obtained from Sigma Chemical Co. Just prior to use, the enzyme suspension was centrifuged at 12,000 *g* for 45 min at 4°C. The supernatant was discarded and the pellet was suspended in 20 mM imidazole buffer, pH 7.4.

Patients

Thirty-five patients admitted to the hospital for elective cholecystectomy were included in the study. Four were males and 31 females; their ages ranged from 30 to 76 yr, with a mean of 51 yr. They had no clinical or laboratory evidence of hepatic or intestinal disease. All patients gave their informed consent to participate in the study according to the declaration of Helsinki. The study was approved by the Ethical Committee of the Karolinska Institute at Huddinge University Hospital.

Experimental procedure

The patients were admitted to the Department of Surgery for elective cholecystectomy 2 days prior to surgery. They were given the regular hospital diet, containing about 0.5 mmol cholesterol per day. All operations were performed between 8 and 9 AM after a 12-h fast. Immediately after opening the abdomen, a liver biopsy (1–4 g) was obtained. A small specimen of the liver biopsy was sent for histological examination, which in all cases was normal. After the biopsy was taken, a regular cholecystectomy was performed without any complications. The major part of the liver biopsy from half of the patients was placed directly in ice-cold homogenizing medium and transported to the laboratory within 10 min for preparation of microsomes. For the other patients the biopsy was first divided into two pieces. One piece was placed in ice-cold homogenizing medium. The other piece was first frozen in liquid nitrogen for at least 30 min before being transferred to ice-cold homogenizing medium.

Preparation of the liver microsomes

The liver biopsies, unfrozen or frozen, were minced and homogenized with a loose-fitting Teflon pestle in nine volumes of 50 mM Tris-HCl buffer, pH 7.4, containing 0.3 M sucrose and 1 mM EDTA. In some experiments, 50 mM NaCl or 50 mM NaF was also included in the presence and absence of protease inhibitors (EGTA, 5.0 mM, PMSF, 1.0 mM, and leupeptin, 50 μM). The homogenate was centrifuged at 20,000 *g* for 15 min. The supernatant was centrifuged at 100,000 *g* for 60 min. The resulting microsomal fraction was suspended in 0.1 M phosphate buffer, pH 7.4, containing 1mM EDTA, to give a final concentration of 10% (w/v). In some experiments, a portion of the microsomal pellet was frozen in liquid N_2 for 30 min followed by thawing at 4°C before suspension in the phosphate buffer solution. The microsomal content of protein was determined by the method of Lowry et al. (10). The concentration of unesterified cholesterol was determined by isotope-mass spectrometry after extraction as described previously (11) and with the modifications previously described (12). The coefficient of variation was 7% (n = 59).

Assay of ACAT activity

Two assay systems were used. Both contained 0.1 ml of the microsomal preparation, corresponding to 0.1–0.2 mg of protein, and 1 mg of defatted human serum albumin in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA, to give a final volume of 1.0 ml. In the first assay system, the mixture was preincubated for 5 min at 37°C in air. The other assay system was preincubated with 50 nmol of exogenous cholesterol (dissolved in 0.6 mg Triton WR-1339) for 20 min at 37°C (13). The reaction was started by the addition of 25 nmol, 1.45 μCi, of [^{14}C]oleoyl coenzyme A. The assay was stopped after 6 min by the addition of 10 ml chloroform–methanol 2:1 (v/v). [^{3}H]cholesteryl oleate, 0.01 μCi, was added as internal standard to estimate recovery, followed by 1 ml 0.9% (w/v) NaCl. The chloroform phase was collected, and evaporated to dryness under N_2. The residue was dissolved in chloroform–methanol 2:1 (v/v), and subjected to thin-layer chromatography together with unlabeled cholesteryl oleate as marker. The chromatogram was developed in hexane–ethyl acetate 95:5 (v/v). The cholesteryl oleate zone was located by iodine vapor and scraped off into a counting vial. A Packard liquid scintillation spectrometer, Model 3003, was used for determining the radioactivity, using Aquasol as scintillator liquid.

The enzyme assay was carried out in duplicate. Each experiment also included two assays in which the amount of [^{14}C]cholesteryl oleate was determined in the incubation mixture extracted at zero-time. The amount of cholesteryl oleate formed during an incubation was estimated by subtracting the amount present in the zero-time assay (always corresponding to less than 0.5 pmol · min^{-1} · mg protein^{-1}) from the amount present at the end of incubation and was expressed as pmol · min^{-1} · mg protein^{-1}.

The activity of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase was assayed as described previously (14).

Statistical procedures

Data are given as means ± SEM. Significances of differences were tested using Wilcoxon's matched-pairs test.

RESULTS

The microsomal fraction obtained from human liver incorporated [^{14}C]oleate into cholesteryl oleate at a rate that was linear with time for at least 10 min (**Fig. 1**), and an incubation time of 6 min was therefore chosen to assure optimal assay conditions. The rate of formation of cholesteryl oleate was proportional to the protein concentration up to at least 0.7 mg per assay (**Fig. 2**). The dependence of ACAT activity on oleoyl CoA concentration was clearly demonstrated. At the concentration of endogenous cholesterol in the microsomes, saturation of the enzyme was obtained at an oleoyl coenzyme A concentration of about 20 μM (**Fig. 3**). The enzyme activity averaged 6.6 ± 0.7 (mean ± SEM) pmol · min^{-1} · mg protein^{-1} in 34 patients undergoing cholecystectomy (**Table 1** and **Table 2**). Three patients, nos. 14, 32 and 34, displayed comparatively higher levels of ACAT activity. No reasonable explanation to that could be found. The patients were normolipidemic, of normal weight, and did not abuse alcohol. They did not suffer from any other disease than cholesterol gallstones.

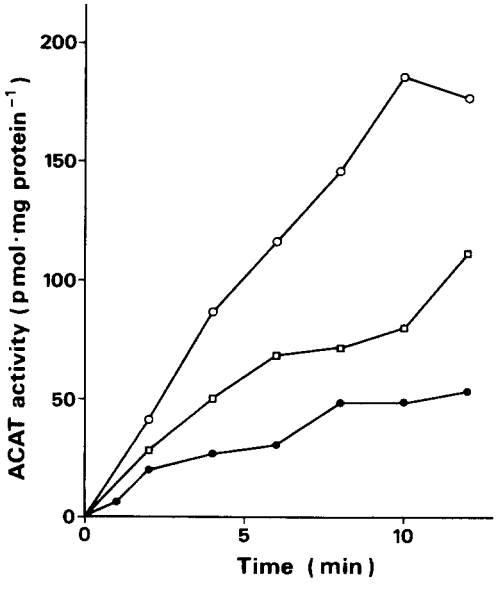

Fig. 1. Effect of incubation time on the ACAT activity in microsomes of unfrozen liver tissue (●), microsomes of frozen liver tissue (○), and microsomes of unfrozen liver tissue preincubated in the presence of exogenous cholesterol (□). With the exception of incubation time, the assay conditions were conducted as described in Materials and Methods.

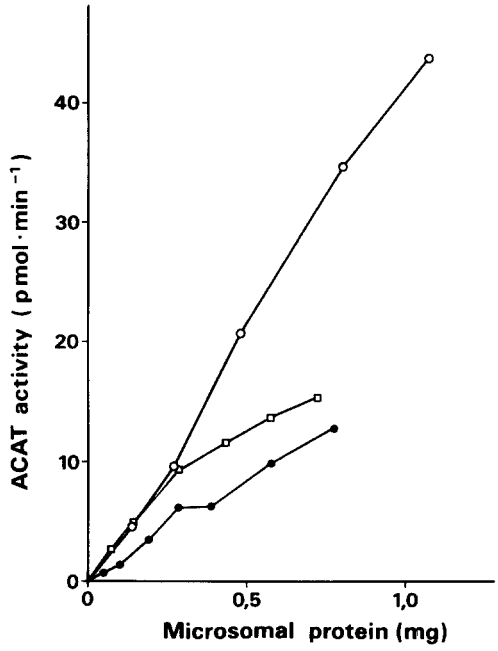

Fig. 2. Effect of microsomal protein on the ACAT activity in microsomes of unfrozen liver tissue (●), microsomes of frozen liver tissue (○), and microsomes of unfrozen liver tissue preincubated in the presence of exogenous cholesterol (□). With the exception of amount of microsomal protein, the assay conditions were conducted as described in Materials and Methods.

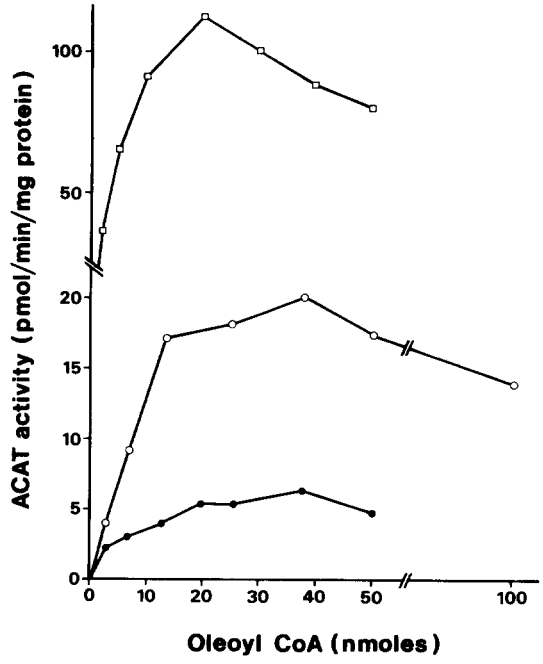

Fig. 3. Effect of different concentrations of [^{14}C]oleoyl CoA on the ACAT activity in microsomes of unfrozen liver tissue (●), microsomes of frozen liver tissue (○), and microsomes of unfrozen liver tissue preincubated in the presence of exogenous cholesterol (□). With the exception of concentration of [^{14}C]oleoyl CoA, the assay conditions were conducted as described in Materials and Methods.

TABLE 1. ACAT activity and pool of unesterified cholesterol in human liver microsomes prepared from unfrozen liver tissue (A) or liver tissue frozen with liquid nitrogen (B)

Patient	ACAT Activity		Unesterified Cholesterol	
	A	B	A	B
	pmol · min⁻¹ · mg protein⁻¹		*nmol · mg protein⁻¹*	
1		30.2		
2	1.6			
3	5.2	14.8	47.8	66.1
4	3.2	18.5	59.4	81.1
5	2.4			
6	4.4	30.0	56.1	82.2
7	4.8	27.2	64.3	103.4
8	2.1	23.0	23.8	42.1
9	5.9	24.9	49.4	70.5
10	5.6	32.2	63.8	64.3
11	3.1	29.0	83.7	80.6
12	9.6	40.8	66.0	68.8
13	3.9	43.6	71.7	96.0
14	19.3	69.4	79.0	82.9
15	4.8	18.8	47.3	62.2
16	9.2	47.7	97.6	114.4
17	6.2	40.8		
Means ± SEM	5.7 ± 1.0	32.7 ± 3.5[a]	62.3 ± 5.2	78.0 ± 5.3[a]

[a]Significantly different from A, $P < 0.001$.

Freezing the liver biopsy in liquid nitrogen for at least 30 min before preparation of microsomes increased the enzyme activity five- to sixfold, from 5.7 ± 1.0 pmol · min^{-1} · mg $protein^{-1}$ to 32.7 ± 3.5 pmol · min^{-1} · mg $protein^{-1}$ (Table 1). In some experiments the microsomal fraction prepared from unfrozen liver tissue was frozen in liquid nitrogen for 30 min followed by thawing at 4°C. This procedure increased the ACAT activity about twofold (**Table 3**). Repeated freezing and thawing up to five times did not further increase the enzyme activity. The addition of protease inhibitors, EGTA, PMSF, and leupeptin, to the buffer in which the liver biopsy was placed and to the microsomal fraction prepared did not influence the stimulatory effect of freezing.

Exogenous cholesterol was added to the incubation mixture dissolved in 0.6 mg Triton WR-1339 as described by Billheimer, Tavani, and Nes (13). Addition of only 0.6 mg Triton WR-1339 inhibited the ACAT activity by about 50% whereas addition of cholesterol dissolved in Triton WR-1339 increased the enzyme activity severalfold. A preincubation time of 20 min was optimal. Saturation of the enzyme was obtained at the addition of 50 nmol of cholesterol (**Fig. 4**). Under these conditions the ACAT activity was increased about fivefold from 7.7 ± 0.8 pmol · min^{-1} · mg $protein^{-1}$ to 36.3 ± 4.6 pmol · min^{-1} · mg $protein^{-1}$ (Table 2). Addition of exogenous cholesterol to frozen microsomes increased the enzyme activity two to three times, to about the same level as that obtained in unfrozen microsomes after addition of cholesterol (Table 3). Addition of exogenous cholesterol to microsomes prepared from frozen liver tissue did not further increase the ACAT activity (**Table 4**).

The concentration of unesterified cholesterol averaged 62.3 ± 5.2 nmol · mg $protein^{-1}$ in microsomes prepared from unfrozen liver tissue (Table 1). Freezing of the

TABLE 2. ACAT activity in human liver microsomes prepared from unfrozen liver tissue, in the absence (A) or presence (B) of exogenous cholesterol

Patient	ACAT Activity	
	A	B
	pmol · min⁻¹ · mg protein⁻¹	
12	9.6	44.8
13	3.9	13.5
14	19.3	111.9
15	4.8	23.8
16	9.2	52.4
17	6.2	32.4
18	5.0	30.4
19	8.2	50.9
20	3.6	23.1
21	7.2	34.9
22	5.2	18.5
23	9.3	48.6
24	5.6	24.9
25	5.5	12.4
26	4.8	23.9
27	4.8	24.1
28	5.6	20.5
29	8.4	26.0
30	5.9	14.8
31	5.6	48.3
32	17.3	34.8
33	9.6	49.1
34	13.6	71.2
35	7.8	44.5
Means ± SEM	7.7 ± 0.8	36.7 ± 4.4[a]

[a]Significantly different from A, $P < 0.001$.

TABLE 3. ACAT activity in unfrozen human liver microsomes and microsomes frozen with liquid nitrogen, assayed in the absence (A) or presence (B) of exogenous cholesterol

Patient	ACAT Activity in Unfrozen Microsomes		ACAT Activity in Frozen Microsomes	
	A	B	A	B
	pmol · min^{-1} · mg protein^{-1}			
10	5.6		13.4	
11	3.1		16.1	
17	6.2	32.4		25.7
28	5.6	20.5	10.5	18.6
29	8.4	26.0	13.8	26.2
30	5.9	14.8	13.9	16.4
31	5.6	48.3		40.3
32	17.3	34.8	35.8	64.2
33	9.6	49.1	12.5	61.3
34	13.6	71.2	18.2	60.0
35	7.8	44.5	10.5	44.2
Means ± SEM	8.1 ± 1.2	38.0 ± 5.4[a]	16.1 ± 2.4[b]	39.7 ± 5.9[c]

[a]Significantly different from A, $P < 0.01$.
[b]Significantly different from unfrozen microsomes, $P < 0.01$.
[c]Significantly different from A, $P < 0.02$.

microsomal fraction did not change the cholesterol concentration. However, in microsomes prepared from frozen liver tissue the concentration of unesterified cholesterol was about 25% higher (78.0 ± 5.3 nmol · mg protein^{-1}) than that of microsomes prepared from unfrozen liver tissue ($P<0.001$) (Table 1).

In order to evaluate whether or not the ACAT activity of human liver microsomes could be influenced by the cytoplasmic phosphatases that are liberated during the subcellular fractionation, microsomes were prepared in the presence and absence of NaF (which inhibits all phosphatase activity). The presence of 50 mM NaF during homogenization of the liver and preparation of the microsomes did not influence the ACAT activity (**Table 5** and **Table 6**). Neither did the presence of NaF influence the stimulatory effect of freezing. Preincubation of the microsomes with 2 mM ATP and 5 mM $MgCl_2$ in phosphate buffer or imidazole buffer for 60 min at 37°C did not have any consistent effect on the enzyme activity (Table 6). Preincubation of the microsomal fraction for 60 min at 37°C in 20 mM imidazole buffer and in the presence of 10 units of *E. coli* alkaline phosphatase did not affect ACAT activity (Table 6).

In contrast, the HMG-CoA reductase activity was lower in microsomes prepared in the presence of NaF compared to those prepared in NaCl (Tables 5 and 6). Preincubation of the microsomes with ATP and $MgCl_2$ inactivated the HMG-CoA reductase, whereas preincubation in the presence of *E. coli* alkaline phosphatase activated the enzyme (Table 6). These results are in agreement with the existence of active and inactive forms of HMG-CoA reductase due to dephosphorylation-phosphorylation (14).

DISCUSSION

The present study confirms and extends previous reports by Balasubramaniam et al. (2) and Erickson and Cooper (3) that human liver microsomes contain ACAT activity. In the absence of exogenous cholesterol, the ACAT activity averaged 6.6 pmol · min^{-1} · mg protein^{-1} (range, 1.6–19.3) in 34 subjects undergoing cholecystectomy. A similar level of ACAT activity was also found by Erickson and Cooper (3) in liver biopsies from three pa-

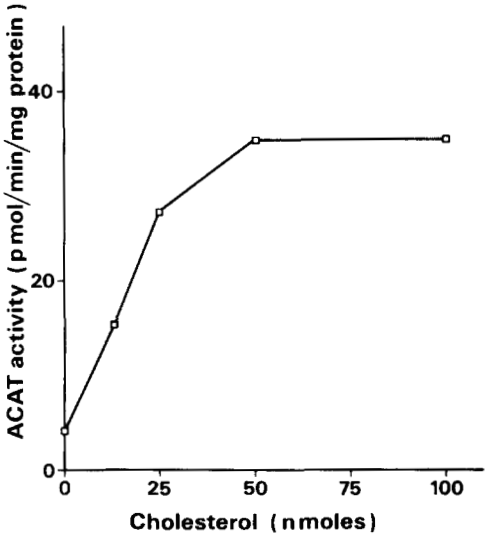

Fig. 4. Effect of preincubation of microsomes of unfrozen liver tissue with different amounts of exogenous cholesterol on the ACAT activity. With the exception of concentration of unlabeled cholesterol, the assay conditions were conducted as described in Materials and Methods.

TABLE 4. ACAT activity in human liver microsomes, prepared from unfrozen liver tissue or liver tissue frozen with liquid nitrogen, in the absence (A) or presence (B) of exogenous cholesterol

Patient	ACAT Activity in Microsomes of Unfrozen Liver Tissue		ACAT Activity in Microsomes of Frozen Liver Tissue	
	A	B	A	B
	pmol · min⁻¹ · mg protein⁻¹			
12	9.6	44.8	40.8	36.2
13	3.9	13.5	43.6	26.9
14	19.3	111.9	69.4	103.1
15	4.8	23.9	18.8	28.9
16	9.2	52.4	47.7	34.5
17	5.2	32.4	40.8	69.7
Means ± SEM	8.8 ± 2.1[a]	46.5 ± 13.0	43.5 ± 6.0	49.9 ± 11.3

[a]Significantly different from other groups, $P < 0.05$.

tients with Hodgkin's disease (range 3.5–18.0 pmol · min^{-1} · mg $protein^{-1}$). On the other hand, Balasubramaniam et al. (2, 15) reported considerably higher ACAT activities in patients with various forms of gastrointestinal diseases, mean values being 26.5 and 23.9 pmol · min^{-1} · mg $protein^{-1}$, respectively.

A major new finding of the present study was that freezing of the liver biopsies in liquid nitrogen consistently increased the ACAT activity five- to sixfold. As Balasubramaniam et al. (2, 15) reported that most of their liver biopsies had been kept frozen at −60°C before microsomal preparation, the comparatively high ACAT activities observed by these authors is probably due to a freezing effect. In fact, the enzyme activities reported by Balasubramaniam et al. were of the same magnitude as those obtained in frozen liver biopsies in the present study.

The mechanism of the freezing effect on the ACAT activity is not known, but several different explanations may be considered. One important factor may be the fact that the microsomal pool of unesterified cholesterol was about 25% larger in the microsomes prepared from frozen liver tissue compared with microsomes prepared from unfrozen liver tissue. Thus, cholesterol was apparently recruited to the microsomal ACAT substrate pool, possibly from plasma membranes. Such recruitment has been suggested to occur under other conditions of enrichment of microsomes with cholesterol (16). It has earlier been claimed that ACAT is not a true rate-determining enzyme, but instead reflects the availability of unesterified cholesterol in the vicinity of the enzyme (17, 18). Disruption of the microsomal membranes by freezing probably also makes the substate(s), the endogenous microsomal cholesterol and/or the ^{14}C-labeled oleoyl CoA, more available to the ACAT enzyme. The fact that freezing of the microsomal fraction prepared from unfrozen liver biopsies also enhanced the ACAT activity without increasing the amount of free cholesterol gives support to this additional explanation.

Another important finding of the present study was that preincubation of the microsomal fraction prepared from unfrozen liver tissue with unlabeled cholesterol increased the ACAT activity about fivefold. Similar results were recently reported by Smith et al. (4) in a small series

TABLE 5. ACAT activity and HMG-CoA reductase activity [a] in human liver microsomes prepared with and without NaF from unfrozen liver tissue (A) or liver tissue frozen with liquid nitrogen (B)

Patient	ACAT Activity in Microsomes Prepared with NaCl, 50 mM		ACAT Activity in Microsomes Prepared with NaF, 50 mM		HMG-CoA Reductase Activity in Microsomes Prepared with NaCl, 50 mM	HMG-CoA Reductase Activity in Microsomes Prepared with NaF, 50 mM
	A	B	A	B	A	A
	pmol · min⁻¹ · mg protein⁻¹					
5	2.4		1.6			
6	4.4	30.0	3.0	21.1		
7	4.5	27.2	4.4	26.7	140.6	16.3
9	5.9	24.9	6.4	17.1	166.3	30.7

[a]HMG-CoA reductase activity was assayed as described recently (14).

TABLE 6. ACAT activity and HMG-CoA reductase activity[a] in unfrozen human liver microsomes prepared with and without NaF

Microsomal Fraction	ACAT Activity				HMG-CoA Reductase Activity
	Patient No. 27		Patient No. 28		Patient No. 27
	A	B	A	B	B
	pmol · min⁻¹ · mg protein⁻¹				
Microsomes prepared in NaCl					
Addition during preincubation					
None	14.6	13.8	5.5	5.9	125.5
APT (2 mM) + $MgCl_2$ (5 mM)	14.1	20.1	5.9	6.8	46.4
Alkaline phosphatase		14.9		6.2	153.6
Microsomes prepared in NaF					
Addition during preincubation					
None	10.5	12.1	5.5	5.7	33.4
APT (2 mM) + $MgCl_2$ (5 mM)	10.4	13.1	5.3	6.0	28.2
Alkaline phosphatase		11.2		5.5	157.6

The microsomes were preincubated in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA (A) or in 20 mM imidazole buffer, pH 7.4, containing 1 mM EDTA (B) in the absence or presence of 2 mM ATP and 5 mM $MgCl_2$ or 10 units of *E. coli* alkaline phosphate for 60 min at 37°C.
[a]HMG-CoA reductase activity was assayed as described recently (14).

of patients. Previous failure to demonstrate that human hepatic ACAT can utilize exogenous cholesterol as a substrate may be explained by the fact that cholesterol was added in acetone (2), i.e., a form that cannot be utilized by ACAT in rat liver (18, 19). Preincubation of the microsomal fraction prepared from frozen liver tissue with exogenous cholesterol, on the other hand, did not further increase the enzyme activity above that obtained in unfrozen liver microsomes, indicating that the enzyme was already saturated with substrate. These results further support the contention that the freezing procedure makes more endogenous cholesterol available for the ACAT enzyme.

Recently it has been reported that the activity of ACAT in rat liver may be subject to short-term regulation by changes in its phosphorylation state (5–9). However, in the present work no evidence was obtained to support the concept that ACAT is activated-inactivated by phosphorylation–dephosphorylation. The present experiments were conducted using conditions almost identical to those under which the human HMG-CoA reductase was inactivated-activated. It should be mentioned that we did not use liver cytosols in our experiments as has been done by the authors that reported on possible phosphorylation–dephosphorylation of ACAT (5–9). Therefore, we cannot completely exclude the possibility that the discrepancy was due to differences in experimental procedure. Final conclusions regarding regulation of ACAT activity by phosphorylation–dephosphorylation will thus have to await experiments with purified enzyme preparations.

In conclusion, the present study has confirmed that human liver microsomes contain ACAT activity. Immediate freezing of the liver biopsy in liquid nitrogen increased the enzyme activity severalfold. This finding may explain previous disparate results on the ACAT activity reported in the literature. The stimulatory effect of freezing was probably partly due to an expansion of the pool of endogenous cholesterol in the microsomal fraction. Preincubation of the microsomal fraction, prepared from unfrozen liver tissue, with exogenous cholesterol increased the enzyme activity to the same level as did freezing. No evidence was obtained to suggest that ACAT in human liver microsomes is activated-inactivated by phosphorylation–dephosphorylation.

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