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Changes in both acyl-CoA:cholesterol acyltransferase activity and microsomal lipid composition in rat liver induced by distal-small-bowel resection

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The acyl-CoA:cholesterol acyltransferase (ACAT) activity and lipid composition of hepatic microsomal membrane were investigated 6 weeks after both 50 and 75% distal-small-bowel resection (SBR). A significant decrease in hepatic cholesteryl ester levels was observed after SBR, with a significant increase in the cholesteryl ester content of the livers of 75% SBR compared with the 50% SBR. Hepatic total acylglycerols, free cholesterol and phospholipid levels were not modified after the surgical operation. Microsomal free cholesterol was increased after both 50 and 75% SBR. However, a decrease in both microsomal ACAT activity and cholesteryl ester levels were found in microsomes (microsomal fractions) of resected rats, both changes being higher after 75 than after 50% resection. The total phospholipid content of the microsomes did not change after the surgical operation. The microsomal phospholipid fatty acid composition indicated higher changes after 75 than after 50% SBR. These results demonstrated that, in resected animals: (1) the activity of the enzyme responsible for catalysing cholesterol esterification (ACAT) is decreased, and (2) hepatic microsomal free cholesterol does not appear to influence the activity of ACAT.

INTRODUCTION

Microsomal 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (EC 1.1.1.34) and acyl-CoA:cholesterol acyltransferase (ACAT) (EC 2.3.1.26) are the major rate-controlling enzymes in cholesterol biosynthesis and its conversion into cholesterol ester respectively. The interruption of the enterohepatic circulation of bile salts has a well-defined stimulatory effect on cholesterol synthesis and HMG-CoA reductase [1]. We have recently reported that distal-small-bowel resection (SBR) increases the activity of HMG-CoA reductase in rat liver [2]. However, the effect of the interruption of the enterohepatic circulation on the enzyme responsible for catalysing cholesterol esterification (ACAT) is less well established.

Recently, much experimental evidence has been presented concerning membrane lipid composition in the regulation of ACAT activity [3]. The influence of changes in the saturation of dietary fatty acids and cholesterol feeding on ACAT activity has been described by different authors [4,5], but the relationship between the activity of this enzyme and the chain length, the extent of unsaturation of constituent fatty acids and the cholesterol concentration of hepatic microsomal membranes is poorly understood.

On the other hand, the interruption of the enterohepatic circulation modifies the lipid intestinal uptake, and a change in the microsomal lipid composition could have occurred.

The present study was designed to determine the effect of distal SBR on both the ACAT activity and microsomal lipid composition in rat liver 6 weeks after the surgical operation.

EXPERIMENTAL

Materials

All radiochemicals for the assay of ACAT activity were obtained from Amersham International. All other chemicals were from Sigma Chemical Co.

Animals

Male Wistar-strain rats, purchased from Iffa-Credo (Lyon, France) and weighing about 300 g each, were used. The animals were given food (Panreac A-04) and water *ad libitum* and housed in a room maintained at 21 ± 2 °C with lights on from 08.00 to 20.00 h. The composition of the diet was as follows: lipids, 3.5%; protein, 19.0%; starch, 66.0%; non-nutritive cellulose, 5.0%; mineral mix, 5.5%; and vitamin mix, 1.0%. The rats were randomly assigned to one of three groups: sham-operated, 50 and 75% SBR. Operative details were described previously [6]. Briefly, the rats were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (4.5 mg/100 g body wt.) after a 24 h fasting period, laparotomy was performed, and rats assigned for SBR underwent either 50 or 75% SBR by excision of the distal small intestine beginning 1 cm proximal to the ileocaecal junction. Before SBR, the blood vessels of the resected intestinal segment were tied and sectioned and the blood supply and the innervation of the remaining intestine were carefully maintained. Intestinal continuity was re-established by an end-to-end anastomosis. Finally, both muscle and cutaneous layers were sutured separately with appropriate thread. Rats from the sham-operated group underwent simple mid-small-intestinal transection, without removal of any tissue, followed by re-

Abbreviations used: ACAT, acyl-CoA:cholesterol acyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; SBR, small-bowel resection.

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anastomosis. After the surgical operation, the rats were housed in a temperature-controlled laboratory with a strict 04.00–16.00/16.00–04.00 h dark/light circle.

At 6 weeks after SBR, the rats were starved overnight (with access to water only) and then killed by a blow at the base of the skull between 09.00 and 10.00 h, to coincide with the peak nocturnal activity of ACAT of the rat [7]. Both control and experimental groups were treated in the same manner to prevent effects which could mask differences between groups.

Preparation of hepatic microsomes

For the preparation of microsomes (microsomal fractions), the livers were excised, weighed and washed in an ice-cold homogenization medium. All subsequent operations were carried out at 4 °C. Livers were homogenized in a Potter–Elvehjem homogenizer with ice-cold homogenization medium containing 0.25 M-sucrose/10 mM-Hepes (pH 7.4)/20 mM-EDTA/2 mM-EGTA/5 mM-DTT. Each homogenate was centrifugated for 20 min at 15000 *g*. The supernatant was collected and centrifugation (15000 *g*) was repeated. The 15000 *g* supernatant was centrifuged at 105000 *g* for 60 min in a Sorvall ultracentrifuge, model OTD 50B. The resulting microsomal pellets were immediately frozen in liquid N_2 and stored at −70 °C until the assays. The storage time did not result in a significant loss of enzyme activity (results not shown).

ACAT assays

ACAT activity was measured essentially as described by Field & Salome [8] with slight modifications. ACAT activity was estimated by measuring the incorporation of [^{14}C]oleate from [^{14}C]oleoyl-CoA into cholesteryl esters utilizing endogenous cholesterol. This assay was chosen to follow the reaction because the endogenous acyl CoA pool is very small [4,8], and therefore dilution of radiolabelled oleoyl-CoA by endogenous substrate is minimal. Each assay mixture contained 0.2–0.4 mg of microsomal protein in 0.1 M-Tris/HCl/0.25 M-sucrose/1 mM-dithiothreitol/1 mM-EDTA and 1 mg of fatty-acid-poor bovine serum albumin, adjusted to pH 7.4 in a final volume of 0.5 ml, before the addition of the substrate. Between 9×10^4 and 2.2×10^4 d.p.m. of [^{14}C]oleoyl-CoA and 20 nmol of oleoyl-CoA was added. This mixture was shaken for 5 min at 37 °C. The reaction was stopped by the addition of 1 ml of chloroform/methanol (2:1, v/v). The samples were immediately subjected to vigorous agitation and phases were separated by centrifugation. The aqueous phase was then removed and the chloroform phase was dried under N_2. The residue was dissolved in 0.150 ml of chloroform and spotted on to t.l.c. plates layered with silica-gel G in order to separate the lipid components. A solvent system consisting of hexane/diethyl ether/acetic acid (80:10:1, by vol.) was used. Lipids were revealed by exposure of the chromatograms to iodine vapour and identified by comparison with positions of standards. The areas corresponding to cholesteryl esters were scraped off directly into liquid-scintillation vials containing 10 ml of a Triton X-100/toluene scintillation solution. Time-dependence, protein-dependence and optimal substrate concentrations were determined in sham-operated and resected rats before this study was performed. These dependences were similar between groups.

Lipid analysis

Lipids were extracted from the liver homogenates and from microsomal fractions by the method of Folch *et al.* [9]. The lipids were separated by t.l.c. on silica-gel H, using hexane/diethyl ether/acetic acid (80:20:1, by vol.) respectively. After the development on the plate, the solvent was allowed to evaporate. Spots were revealed with iodine vapour and the rows of spots were delineated. Each row of spots (phospholipids, cholesterol, cholesteryl esters and triacylglycerols) were removed from the plate and eluted from the silica gel with 14 ml of either diethyl ether or chloroform/methanol (2:1, v/v) for cholesterol or cholesteryl esters and phospholipids or acylglycerols respectively. Non-esterified cholesterol and cholesteryl esters were assayed individually by the method of Huang *et al.* [10]. Phospholipids and triacylglycerols were estimated by the Vioque & Holman [11] method. For determination of fatty acyl composition, phospholipid was separated from microsomal total lipid by t.l.c. and the fatty acids analysed as their methyl esters by g.l.c. The samples were saponified by heating for 5 min with 5 ml of 0.2 M-sodium methylate and heated again at 80 °C for 5 min with 6% (w/v) H_2SO_4 in anhydrous methanol. The fatty acid methyl esters thus formed were eluted with hexane and analysed with a gas chromatograph (Hewlett–Packard, model 5710A) equipped with a flame-ionization detector. SP-2310 (3%) and SP-2300 (2%) on 100/120 Chromosorb WAW were used in a 200 cm glass column, and N_2 (20 ml/min.) served as the carrier gas. The temperature was programmed to rise from 190 to 220 °C at a rate of 2 °C/min. Fatty acid methyl esters were identified by comparison of their retention times against those of standards.

Protein assay

Microsomal protein was determined by the method of Lowry *et al.* [12] with bovine serum albumin as the standard.

Statistical analysis

The results shown are means ± S.D. The effect of intestinal resection was examined by analysis-of-variance procedures. The unpaired Student's *t* test was used to test the significance of the difference between the means for sham-operated and resected rats.

RESULTS

Observations in the whole animal

Post-operative mortality was 10 and 20% after 50 and 75% SBR respectively. Deaths occurred within the first 5 post-operative days and were attributed to the surgery. Table 1 shows food consumption, both body and liver weights and liver lipid content for both control and resected rats. All groups of animals consumed the same amount of food, irrespective of some removed small-intestine length. Initial body weights in each group of animals were the same. At the time of the study, 6 weeks after the surgical operation, mean body weights were significantly lower in both 50- and 75%-resected rats compared with sham-operated rats, this decrease being higher after 75 than after 50% SBR. Liver weight, expressed per g of tissue, did not change after the operation. However, the intestinal resection significantly increased this parameter when it was expressed per

Table 1. Food consumption, body weight, liver weight and liver lipid content in sham-operated and resected rats

Results are means ± S.D. for ten animals in each group. Significance: [a] $P < 0.05$; [b] $P < 0.001$ (50%- or 75%-resected animals compared with sham-operated animals); * $P < 0.005$; ** $P < 0.001$ (75%- compared with 50%-resected animals).

Parameter	Sham-operated	50% SBR	75% SBR
Food consumption (g/day)	21 ± 1.5	24 ± 1.8	21 ± 0.7
Body weight (g)			
At entry	306 ± 2	306 ± 7	302 ± 8
At study	387 ± 13	354 ± 10[a]	249 ± 12[b,**]
Liver weight			
(g)	10.5 ± 0.4	10.9 ± 0.4	9.6 ± 0.4
(g/100 g body wt.)	2.72 ± 0.08	3.08 ± 0.14[a]	3.87 ± 0.19[b,*]
Liver lipid content (%)	7.08 ± 0.9	8.4 ± 3.4	9.3 ± 1.3

Table 2. Influence of intestinal resection on the ACAT activity of rat liver microsomes

Results are given as means ± S.D. for eight separate microsomal preparations from eight different rats. Significance: [a] $P < 0.01$; [b] $P < 0.001$ (50%- or 75%-resected animals compared with sham-operated animals); * $P < 0.05$ (75%-compared with 50%-resected animals).

	Sham-operated	50% SBR	75% SBR
ACAT activity (pmol min per mg)	112 ± 8	85 ± 6[a]	68 ± 5[b,*]

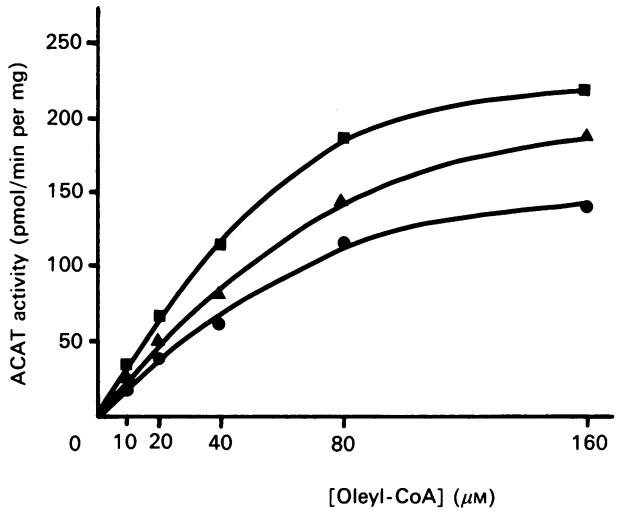

Fig. 1. Effect of oleoyl-CoA concentration on ACAT activity

The microsomal protein content was 0.2–0.4 mg and the incubation time was 5 min. Microsomes were obtained from five separate microsomal preparations from five different rats. ■, Sham-operated; ▲, 50% SBR; ●, 75% SBR.

Table 3. Effect of intestinal resection on liver lipid content

Results are given as means ± S.D. for six animals in each group. Significance: [a] $P < 0.05$; [b] $P < 0.01$ (50%- or 75%-resected rats compared with sham-operated animals); * $P < 0.01$ (75%- compared with 50%-resected animals).

Lipid	Liver lipid (mg/g)		
	Sham-operated	50% SBR	75% SBR
Free cholesterol	1.7 ± 0.08	1.5 ± 0.15	1.7 ± 0.25
Cholesteryl ester	0.8 ± 0.18	0.12 ± 0.02[b]	0.29 ± 0.03[a,*]
Phospholipid	45.7 ± 2.0	42.9 ± 3.4	47.5 ± 6.6
Triacylglycerols	9.9 ± 1.1	7.9 ± 2.7	9.9 ± 0.4

100 g body weight, mainly in 75%-resected rats. This increase was due to the observed weight loss after SBR. On the other hand, animals with SBR did not develop fatty livers, since liver lipid content did not significantly change after the operation.

ACAT activity

A significant decrease in the hepatic activity of the enzyme ACAT was observed after distal SBR, this decrease being higher after 75 than after 50% SBR (Table 2). Fig. 1 depicts the effect of substrate concentration on ACAT activity for the different groups of animals. The differences observed in ACAT activities among the different groups were maintained over this substrate concentration range. The optimal substrate concentrations were similar between control and resected rats.

Liver and microsomal lipid concentrations

In the present studies non-esterified cholesterol, total acylglycerols and phospholipid levels in total liver lipid were similar between sham-operated and resected rats. However, a significant decrease in cholesteryl ester levels was observed after SBR, with a significant increase in the cholesteryl ester content of the livers of 75%-SBR animals compared with the 50% SBR (Table 3).

Analysis of the microsomal lipid composition in these studies demonstrated that most of the cholesterol recovered from the microsomal membrane of sham-operated and resected rats was non-esterified. Non-esterified-cholesterol levels were significantly increased after SBR, whereas a decrease in the esterified-cholesterol content was observed in microsomes from resected rats. Both changes in non-esterified cholesterol and esterified cholesterol content were greater after 75 than after 50% SBR (Table 4). Total phospholipid content of the microsomes did not change after the surgical operation. Therefore the increase in non-esterified-cholesterol content found in resected animals was associated with an enhancement in the molar cholesterol/phospholipid ratio (Table 4).

Table 4. Effect of intestinal resection on microsomal lipid composition

Results are given as means ± S.D. for six separate microsomal preparations from six different rats. Abbreviation: NEC, non-esterified cholesterol. Significance: [a] $P < 0.01$; [b] $P < 0.005$; [c] $P < 0.001$ (50%- or 75%-resected rats compared with sham-operated animals); * $P < 0.05$; ** $P < 0.001$ (75%- compared with 50%-resected animals).

Lipid	Microsomal lipid (nmol/mg of microsomal protein)		
	Sham-operated	50% SBR	75% SBR
NEC	34.18 ± 3	60.97 ± 6.5[b]	102.1 ± 10[b,*]
Cholesteryl ester	6.77 ± 0.46	4.65 ± 0.31[a]	0.60 ± 0.01[c,**]
Phospholipid (PL)	473 ± 90	462 ± 73	405 ± 0.6
NEC/PL	0.072	0.15	0.25

Table 5. Effect of intestinal resection on major fatty acid composition of microsomal phospholipid

The sum of the saturated (sat.), monoenoic (mono.) and polyenoic (poly.) was calculated for all positively identified fatty acids. Results are given as means ± S.D. for six individual preparations from six control and six resected rats. Abbreviation: n.d., not detected; Significance: [a] $P < 0.05$; [b] $P < 0.025$; [c] $P < 0.01$; [d] $P < 0.005$; [e] $P < 0.001$ (50%- or 75%-resected rats compared with sham-operated animals).

Fatty acids	Composition (w/w)		
	Sham-operated	50% SBR	75% SBR
$C_{16:0}$	16.76 ± 2.49	16.23 ± 0.30	9.44 ± 1.40
$C_{16:1,n-7}$	1.07 ± 0.40	1.40 ± 0.08	1.93 ± 0.08[b]
$C_{18:0}$	22.20 ± 0.74	22.80 ± 0.69	12.10 ± 3.70
$C_{18:1,n-9}$	9.09 ± 0.54	9.06 ± 0.23	11.66 ± 0.83[b]
$C_{20:1,n-9}$	0.27 ± 0.01	0.146 ± 0.004[d]	0.13 ± 0.01[d]
$C_{20:3,n-9}$	n.d.	n.d.	0.660 ± 0.004
$C_{18:2,n-6}$	12.65 ± 0.31	11.96 ± 0.46	10.70 ± 0.05[c]
$C_{18:3,n-6}$	n.d.	0.50 ± 0.02	0.860 ± 0.002
$C_{20:3,n-6}$	0.14 ± 0.01	0.15 ± 0.01	0.260 ± 0.003[c]
$C_{20:4,n-6}$	28.44 ± 1.50	29.41 ± 0.61	20.42 ± 2.36[d]
$C_{22:4,n-6}$	0.62 ± 0.11	0.28 ± 0.02[e]	0.25 ± 0.02[e]
$C_{22:5,n-6}$	0.62 ± 0.12	0.41 ± 0.03[a]	0.38 ± 0.04[b]
$C_{18:3,n-3}$	n.d.	0.25 ± 0.12	0.110 ± 0.004
$C_{22:5,n-3}$	0.92 ± 0.12	0.64 ± 0.09[b]	0.36 ± 0.04[d]
$C_{22:6,n-3}$	5.22 ± 1.32	3.36 ± 0.21[a]	2.81 ± 0.03[c]
Sat.	38.96 ± 3.23	39.03 ± 0.99	40.54 ± 5.10
Mono.	10.43 ± 0.94	10.61 ± 0.31	13.70 ± 0.91[b]
Poly.	59.03 ± 4.43	57.60 ± 1.89	50.53 ± 2.48[c]
Total $n-6$	42.46 ± 3.48	42.71 ± 1.16	32.87 ± 2.50[c]
Total $n-3$	6.14 ± 1.44	4.25 ± 0.42[b]	3.28 ± 0.07[c]
$C_{20:4}/C_{18:2}$ ratio	2.25	2.46	1.91

Microsomal fatty acid composition

Analysis of the major fatty acid composition of microsomal phospholipid is shown in Table 5. Saturated fatty acids were not significantly different among the groups. An increase in monounsaturated fatty acids ($C_{16:1}$ and $C_{18:1}$), and a decrease in polyunsaturated fatty acids were only obtained after 75% SBR. Regarding the $n-6$ fatty acids, a decrease in the levels of $C_{18:2,n-6}$ and $C_{20:4,n-6}$ fatty acids was observed in 75%-resected rats, accompanied by a reduction in the ratio of $C_{20:4}/C_{18:2}$ fatty acids. However, $C_{22:4,n-6}$- and $C_{22:5,n-6}$-fatty-acid levels were diminished after both 50% and 75% SBR. The $C_{20:3,n-6}$-fatty-acid levels were only increased after 75% SBR. Therefore a decrease in total $n-6$ fatty acids was obtained in 75%-resected animals. In the case of $n-3$ fatty acids, intestinal resection reduced the levels of $C_{22:5,n-3}$ and $C_{22:6,n-3}$. These levels are responsible for the lower value in total $n-3$ fatty acids found in resected animals in comparison with those in sham-operated rats. For the $n-9$ fatty acids, a reduction in $C_{20:1,n-9}$ fatty acids was observed after SBR. The $C_{20:3,n-9}$ fatty acid was only found in detectable levels in microsomes of 75%-resected rats.

DISCUSSION

The interruption of the enterohepatic circulation produces a decrease in the input of bile acids into the liver, leading to an increase in hepatic synthesis of both bile acids and cholesterol. Thus we have recently found an increase in both the total activity and activity state of the enzyme HMG-CoA reductase in rat liver after distal SBR [2]. However, the effect of distal SBR on ACAT activity is unknown. Our results show that intestinal resection produces a decrease in the specific activity of ACAT, this decrease being higher after 75 than after 50% distal SBR (Table 2).

In the present studies, non-esterified cholesterol, total triacylglycerols and phospholipid in total liver lipid did not change after distal SBR. However, a decrease in cholesteryl ester levels in liver lipid was observed after SBR, this decrease being higher after 50 than after 75% distal SBR (Table 3). Therefore, no consistent relationship was found between liver cholesterol levels and enzyme activity.

Analysis of the microsomal lipid composition demonstrated that the decrease in ACAT activity after SBR was accompanied by an increase in non-esterified-cholesterol levels in 50- and 75%-resected rats (Table 4). As a consequence, a positive association between ACAT activity and the microsomal non-esterified cholesterol does not appear to exist after distal SBR. Similar observations had been demonstrated previously in cholestyramine (bile-acid-binding resin)-treated rats [7]. These findings are not in agreement with previous reports indicating a positive association between ACAT activity and microsomal non-esterified cholesterol under normal physiological conditions [7,13]. However, in both 50- and 75%-resected animals, a decrease in microsomal cholesteryl ester levels, together with a simultaneous decrease in hepatic activity in ACAT, were observed. The total phospholipid content of the microsomes did not change after the surgical operation. Thus the increase in non-esterified-cholesterol content after resection was associated with an enhancement in the molar cholesterol phospholipid ratio of microsomal membranes of resected rats (Table 4).

The increased hepatic microsomal non-esterified cholesterol observed after distal SBR was not paralleled by an increase in hepatic cholesterol levels. These results

might suggest that some transport process is also affected, so that cholesterol does not have the ability to be transported to the plasma membrane (the cellular compartment thought to contain the vast majority of the cellular cholesterol).

Alteration of fatty acid composition of microsomal phospholipid as a result of SBR has not been previously reported. Table 5 shows some of the major individual fatty acids. Fatty acid compositional changes have been shown to influence the activity of many enzymes that are tightly bound to membranes. Thus, previous reports have found that the fatty acid composition of microsomal phospholipid, independent of microsomal cholesterol, regulates hepatic ACAT [5,14]. However, the changes in fatty acid composition are relatively small; therefore the observed decrease in ACAT activity in the present study could not be solely attributed to changes in the fatty acyl composition.

Animals with SBR grew less than those without resection. The amount of food eaten per day was similar between groups. Therefore, lower body weight after distal SBR could not be due to difference in energy intake, and might relate to changes in feed efficiency. It is well known that the enterohepatic circulation produces lipid malabsorption [15]. Fat malabsorption is likely to derive from a combination of effective loss of absorptive surface area associated with a severely compromised enterohepatic circulation, which could lead to decreased efficiency of the remaining functional intestine and a decrease in growth rate. Accordingly, any change in ACAT activity may reflect this poor nutritional status in resected animals.

On the other hand, it has been postulated that phospholipid containing arachidonic ($C_{20:4,n-6}$) or linoleic ($C_{18:2,n-6}$) acid is crucial for stable lipoprotein formation [16] and, with a reduced supply of these phospholipids in 75%-resected animals, lipoprotein synthesis might be curtailed and the amount of cholesterol secreted in hepatic lipoproteins would decrease. The amount of dietary lipoproteins delivering cholesterol to the liver also would decrease with significant SBR. Finally, the amount of cholesterol secreted in bile would also be decreased [17]. Therefore, in addition to cholesterol being shunted into new bile-acid synthesis, other possible causes could contribute to an explanation of the decrease in ACAT activity after SBR, such as lipoprotein and biliary cholesterol changes.

As a side result, a decreased $C_{20:4}/C_{18:2}$-fatty-acid ratio observed in rats with 75% SBR might suggest a decreased Δ^5-and Δ^6-desaturase activity [18], although fatty acid malabsorption could not be ruled out.

The significant increase in the cholesteryl ester content of the livers of 75%-SBR animals compared with the 50%-SBR animals is an unexpected finding, especially as the cholesteryl ester content of the microsomes derived from the 75%-SBR rat is considerably lower than that derived from the 50%-SBR rat. Furthermore, these results appear to be inconsistent with the decreased ACAT activity in the 75%-SBR as compared with the 50%-SBR rats. At present there appears to be no reasonable explanation for these findings.

In summary, the results of the present study demonstrate that, in resected animals: (1) the ACAT activity is decreased, and (2) hepatic microsomal non-esterified cholesterol does not appear to influence the activity of ACAT.

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