Serum Cholesterol Esterification in Liver Disease. Combined Determinations of Lecithin: Cholesterol Acyltransferase and Lipoprotein-X

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Received: February 24, 1972, and in revised form: April 10, 1972

Abstract. Indirect evidence suggests that lecithin:cholesterol acyltransferase (LCAT) is synthesized in the liver. There are, however, controversial reports in the literature correlating LCAT activity with various forms of liver disease. The purpose of this study was to determine LCAT activity in icteric patients. These were classified not only by the conventional methods, but also with respect to the presence or absence of LP-X, an abnormal plasma lipoprotein, highly specific for demonstrating or excluding cholestasis. LCAT was measured at different stages of the disease. The patients were divided into 4 groups: I. Hepatitis with cholestasis (LP-X pos.) [17]; II. Hepatitis without cholestsis (LP-X neg.) [12]; III. Extrahepatic biliary obstruction (LP-X pos.) [10]; IV. Chronic liver failure (LP-X neg./pos.) [11]. Compared to normal controls [15], patients from group I had low, group II had normal, group III had normal and group IV had low LCAT activity. *In vitro* studies clearly excluded the existence of circulating inhibitors of LCAT. From these data it is suggested that the ratio of esterified to unesterified cholesterol in liver disease depends on two factors: LCAT activity and the occurrence of the abnormal lipoprotein-X. Furthermore these results indicate that combined determinations of LCAT and LP-X may prove to be useful techniques for differentiating intra- and extrahepatic cholestasis.

Key words: LCAT, LP-X, lipids in liver disease.

In normal human plasma approximately two thirds of the serum cholesterol is esterified [1]. In patients with liver disease, however, the percentage of cholesterol which is esterified may vary to a considerable extent and is often lower than in normals. The reason for this altered ratio of esterified to unesterified cholesterol in liver disease is not fully understood. It has recently been proposed by several investigators [2–6] that low lecithin: cholesterol acyltransferase (LCAT) activity in plasma may be responsible for this characteristic lipid pattern. In agreement with earlier studies by Turner *et al.* [7] these authors demonstrated that this serum enzyme which catalyzes the transfer of fatty acids from the β position of lecithin to the 3-β-OH group of cholesterol [8, 9] is reduced in almost all liver diseases. The question arises, however, whether LCAT alone is responsible for the fall in the ester: free ratio especially in the light of recent findings by several investigators [10–13] who demonstrated an abnormal low density lipoprotein designated lipoprotein-X (LP-X), which can only be detected in plasma of patients with cholestasis [14]. It is now well known that the plasma low density lipoproteins (LDL) in biliary obstruction are characterized almost exclusively by the presence of two immunochemically distinct lipoproteins, lipoprotein-B (LP-B) and lipoprotein-X (LP-X). LP-X has an uniquely high content of unesterified cholesterol and phospholipids and is thereby at least partially responsible for the plasma lipid alterations in obstructive jaundice [12—13]. The immunochemical detection of this abnormal lipoprotein has been found to be specific for any form of cholestasis [18]. And thus this test proved to be very useful in differential diagnosis of liver disease [14]. It seemed reasonable therefore to determine LCAT and LP-X in parallel in patients with various forms of liver disorders. In this study LCAT was assayed in icteric and non-icteric patients who were classified not only by the conventional methods and laboratory data but also with respect to the presence or absence of LP-X. From these results it becomes apparent that the ratio of esterified to unesterified cholesterol in liver disease probably depends on two factors: LCAT activity and the occurrence of the abnormal lipoprotein-X. Furthermore these results indicate that parallel determinations of LCAT and LP-X may prove useful for differentiation between intra- and extrahepatic cholestasis.

* Dr. H. Wengeler participated in this study as a postdoctoral fellow of the Department of Internal Medicine, University of Heidelberg, Germany. Supported by the Deutsche Forschungsgemeinschaft.

Materials and Methods

Patients

Lecithin:cholesterol acyltransferase (LCAT) activity in serum was measured in a control group of 15 healthy volunteers (aged 20 to 49 years). None of these volunteers had abnormal liver function tests and both their lipid levels as well as their lipoprotein patterns were within the normal range. 52 patients with either biliary obstruction or parenchymal liver disease were included in the study. 17 patients were hospitalized for hepatitis with cholestasis, 12 for hepatitis without cholestasis, 10 for extrahepatic biliary obstruction and 11 for chronic liver failure. Diagnoses were confirmed by liver biopsy and/or operation. Clinical data from all patients are summarized in Tables 1–4.

Enzyme Assay

LCAT activity was assayed by measuring the ability of serum to esterify labelled substrate cholesterol. The method has recently been described in detail by Kattermann and Wolfrum [15]. I. Pooled human plasma (pH 7.5) heated for 20 min. at 56°C to inactivate LCAT activity served as the substrate. The free cholesterol concentration was adjusted to 30 mg/100 ml plasma by dilution with 0.9% NaCl. Aliquots of this plasma were stored at −15°C and were used during this study. II. 26-^{14}C-cholesterol (NEN Chicago, spec. activity 57 μC/μMol) was dispersed in a solution of human albumin (Behring Werke, Marburg, Germany) as described by Porte and Havel [16]. Radioactivity was 0.5 μC ^{14}C-cholesterol per ml stock solution. Incubations were done in 10 ml Dole tubes. Each vial contained: (1) 350 μl of solution I, (2) 100 μl of solution II and (3) 50 μl serum as the enzyme source. All samples were run in duplicate. Incubations were done at 37°C for 6 h in a metabolic shaker. Total lipids were extracted as described by Dole [17] and separated by thin layer chromatography (petrolether/ether/acetic acid = 80/20/1 v/v). The areas containing cholesterol and cholesterol esters were visualized by exposure to iodine vapours and scraped into counting vials after the sublimation of iodine. Radioactivity was determined in a liquid scintillation spectrometer (Packard Tri Carb Modell 3380) equipped with external standard for quenching correction. LCAT activity was expressed as n Mol 26-^{14}C-cholesterolester/ml/h × 10^{-2}. Agreement between paired samples was always >'94%.

Blood Chemical Tests

Lipoprotein-X (LP-X) determinaitons were performed by the method of Seidel [18], determinations of Australia Antigen (AuAg) by the method of Shuman *et al.* [19]. Triglycerides (TG) were measured fluorometrically with a Technicon Auto Analyzer (Technicon Instruments Corporation, Channcey, N.Y., U.S.A.) using the standard technique of Kessler and Lederer [20]. Total cholesterol (CHOL), cholesterolester (CE) and free cholesterol (FC) were measured by the method of Sperry and Webb [21] after separation by thin layer chromatography. Lipoprotein electrophoresis was performed as described by Greten *et al.* [22]. Serum transaminases GOT and GPT, and alkaline phosphatase were measured by standard techniques using the LKB Ultrolab System 8600 Reaction Rate Analyzer (LKB Produkter AB, S-16125 Bromma 1 Sweden). Total bilirubin determinations were performed using a Technicon Auto Analyzer by standard techniques.

Normal Values for Blood Chemical Tests

(Chemical laboratory, Department of Internal Medicine, University of Heidelberg).

GOT	< 12 mU/ml
GPT	< 12 mU/ml
Bilirubin	< 1.0 mg/100 ml
Alk. Phos.	< 48 mU/ml

Results

The total radioactive cholesterol esterified in 6 h was directly proportional to the amount of added plasma over a range of 0.01–0.1 ml (Fig. 1). For subsequent assays 0.05 ml of plasma were used routinely. The enzyme reaction proceeded linearly for about 6 h (Fig. 1). No significant differences in LCAT activity were found when serum from two normals was determined three times a day over a seven day period. In 15 healthy control subjects with normal liver function tests serum LCAT activity ranged from 5.6–11.2 n Mol ^{14}C-cholesterol ester/ml/h × 10^{-2} (Fig. 2). Any patient with LCAT activity lower than the lowest normal was defined as having low LCAT activity.

Patients with various forms of liver disease were divided into four groups (Fig. 3). Clinical and laboratory findings of these patients are summarized in Table 1–4. Group I: 17 patients had acute hepatitis and cholestasis as defined by a positive LP-X immunochemical reaction. Group II: 12 patients had acute hepatitis without cholestasis (LP-X negative). Diagnosis in all patients from groups I and II were confirmed by liver biopsy. Group III: 10 patients had extrahepatic biliary obstruction and were also LP-X positive. In all these patients diagnoses were confirmed by laparotomy. Group IV: 11 patients with chronic liver failure. Three of these were LP-X positive, the others had no evidence for cholestasis as demonstrated by a negative LP-X reaction. Most of these patients had liver cirrhosis. Diagnoses were also confirmed by liver biopsy. Fig. 3 summarizes LCAT activity in all patients of these various groups. Compared to normal controls all patients in group I had low LCAT activity, patients in group II had normal LCAT activity, patients from group III had normal and patients from group IV had low LCAT activity. When free and esterified cholesterol were determined in these groups a mean esterified /total cholesterol ratio of 30% in group I, 61% in group II and 45% in group III was found.

Statistical Calculations

Analysis of variance for a one-way design was done on the LCAT activity in the four groups: hepatitis with cholestasis (group I), hepatitis without cholestasis (group II), extrahepatic biliary obstruction (group III) and chronic liver failure (group IV)

Group	Size	Mean	Standard deviation
I	17	0.3818	0.0902
II	12	0.7483	0.1003
III	10	0.7110	0.1436
IV	11	0.2900	0.1239

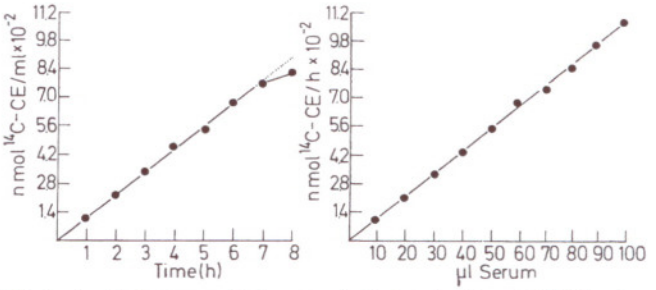

Fig. 1. Esterification of 26-^{14}C-Cholesterol during a 6 hour period. Incubation at 37° C using normal serum (left). Esterification of 26-^{14}C-Cholesterol with increasing amounts of normal serum at 37°C (right)

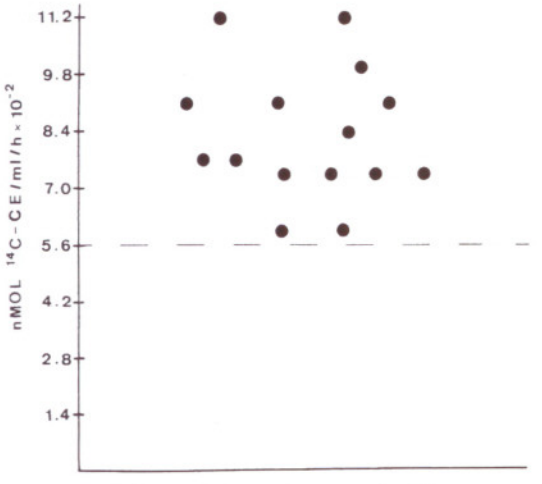

Fig. 2. LCAT activity in 15 normal subjects

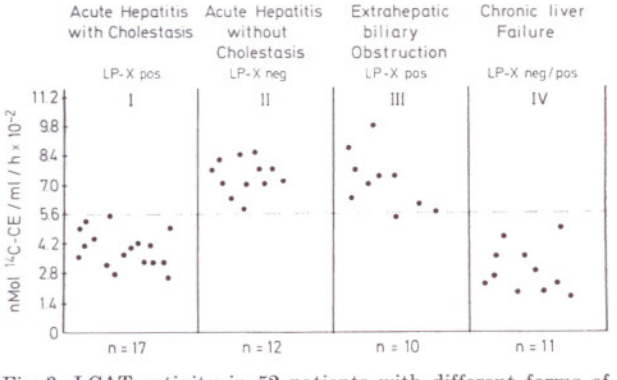

Fig. 3. LCAT activity in 52 patients with different forms of liver disease (group I–IV)

Analysis of Variance

	Sums of squares	DF	Mean squares	F-Ratio
Between groups	1.8962	3	0.6321	50.1530
Within groups	0.5797	46	0.0126	
Total	2.4759	49		

The F-Ratio means $\alpha < 0.001$.

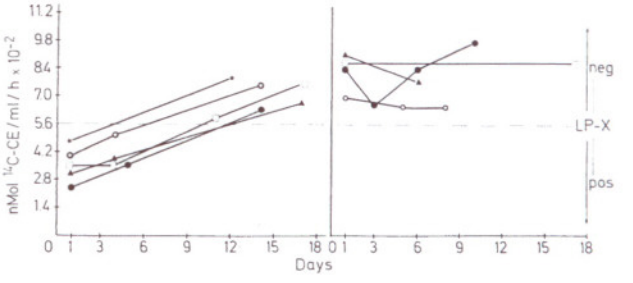

Fig. 4. *Left:* LCAT activity in patients with hepatitis and cholestasis (group I) during follow-up study. *Right:* LCAT activity in patients who had hepatitis without intrahepatic cholestasis (group II) during follow-up study. Patients with LCAT activity < 5.6 nMol ^{14}C-CE/ml/h $\times 10^{-2}$ were LP-X positive. Patients with LCAT activity > 5.6 (above the broken line) were LP-X negative

Furthermore the linear regression was performed for free cholesterol/LCAT activity in groups I and II.

With cholesterol as independent varialbe x and LCAT as response variable y, regression analysis indicated no association between x and y. The regression coefficient $b_1 = -0.02$ was not true in the respective population.

Analysis of Variance for the Regression

	Sums of squares	DF	Mean sqaures	F-Ratio
Regression	12.56069	1	12.56069	3.04131
Deviation of reg.	99.12070	24	4.13003	
Total	111.68138	25		

The F-Ratio means $\alpha = 0.05$.

From these statistical calculations it becomes apparent that the difference between groups is greater than within groups ($\alpha < 0.001$). Thus group I is independent from group III. Furthermore the statistical analysis indicates that we could not prove a linear regression between free cholesterol and LCAT activity ($b_1 = -0.02$) in group I and II. With $\alpha = 0.05$ a linear regression might be possible, however, if either a larger group of patients and/or a second parameter were available.

Table 1. Clinical data from 17 patients with hepatitis and intrahepatic cholestasis. LCAT activity expressed as n Mol ^{14}C-Cholesterolester/ml/h $\times 10^{-2}$

No.	Age	Sex	LCAT	LP-X	TG	Chol	CE/FC	% CE	GOT	GPT	Alk. Phos.	Bilirubin	AuAg
					mg/100 ml				mU/ml		mU/ml	mg/100 ml	
1	33	♂	4.8	+	460	216	53/161	25	403	310	36	16.0	+
2	70	♂	3.4	+	156	156	37/119	23	456	600	68	13.0	+
3	22	♂	5.0	+	255	168	84/ 84	50	224	290	82	12.0	—
4	47	♂	3.9	+	118	119	47/ 72	39	228	240	41	4.5	+
5	60	♀	4.6	+	204	143	44/ 99	30	564	528	78	10.8	+
6	50	♂	3.5	+	125	138	61/ 77	44	100	50	58	2.8	+
7	20	♂	5.6	+	165	216	72/144	30	152	258	56	3.2	—
8	23	♀	2.4	+	172	183	47/136	25	360	580	70	2.5	—
9	32	♀	3.5	+	75	230	39/191	17	128	180	71	11.2	—
10	22	♂	3.8	+	305	210	88/122	42	696	480	84	10.0	—
11	28	♂	4.2	+	205	184	34/150	18	480	204	92	5.2	—
12	19	♂	3.4	+	112	116	37/ 79	32	720	492	68	10.5	+
13	65	♀	3.7	+	379	344	98/246	28	336	260	90	15.0	—
14	32	♂	3.1	+	200	325	69/256	21	626	528	68	6.8	—
15	70	♀	3.2	+	176	237	108/129	46	94	88	162	6.6	—
16	59	♂	2.2	+	158	121	44/ 77	36	24	9	44	2.9	—
17	25	♂	4.6	+	130	160	93/ 67	58	75	160	46	5.5	—

Table 2. Clinical data from 12 patients with hepatitis without intrahepatic cholestasis

No.	Age	Sex	LCAT	LP-X	TG	Chol	CE/FC	% CE	GOT	GPT	Alk. phos.	Bilirubin	AuAg
					mg/100 ml				mU/ml		mU/ml	mg/100 ml	
1	41	♂	7.7	—	155	167	112/ 55	67	27	44	40	0.8	—
2	31	♂	8.0	—	160	201	126/ 75	62	31	54	52	1.8	+
3	20	♀	7.2	—	130	148	98/ 50	66	17	25	33	1.0	—
4	36	♀	5.7	—	260	216	119/ 92	56	264	324	80	6.0	—
5	18	♂	8.4	—	80	154	88/ 66	57	160	120	68	1.0	—
6	35	♂	6.9	—	112	204	129/ 75	62	492	240	80	0.6	—
7	70	♀	8.8	—	150	218	136/ 82	63	130	120	52	0.8	—
8	30	♂	5.9	—	60	102	63/ 39	61	45	27	13	1.1	—
9	30	♂	8.8	—	210	259	135/124	52	90	52	80	3.8	—
10	27	♀	7.0	—	50	136	86/ 50	63	110	125	37	0.7	—
11	64	♀	7.7	—	178	352	215/137	61	72	68	80	5.0	—
12	65	♂	7.7	—	200	240	156/ 84	65	24	36	71	0.9	—

Table 3. Clinical data from 10 patients with extrahepatic biliary obstruction

No.	Age	Sex	LCAT	LP-X	TG	Chol	CE/FC	% CE	GOT	GPT	Alk. phos.	Bilirubin	AuAg
					mg/100 ml				mU/ml		mU/ml	mg/100 ml	
1	42	♀	8.3	+	130	234	137/ 97	58	45	100	66	8.0	—
2	65	♀	6.45	+	155	331	128/203	39	82	133	180	10.3	—
3	38	♂	7.7	+	205	348	156/192	45	108	168	120	3.6	—
4	38	♂	7.0	+	223	272	128/144	47	128	130	220	7.7	—
5	45	♀	9.9	+	143	193	95/ 98	50	120	172	96	10.0	—
6	79	♀	7.1	+	143	350	182/168	52	120	172	96	10.0	—
7	63	♀	5.2	+	250	505	81/424	16	80	260	210	13.0	—
8	70	♂	7.3	+	160	245	99/146	40	25	19	150	8.0	—
9	65	♂	6.0	+	170	211	75/136	35	15	12	180	5.0	—
10	50	♀	7.0	+	130	201	129/ 72	64	52	68	130	5.5	—

5 patients with acute hepatitis and cholestasis (group I) were studied serially. As seen on Fig. 4 these patients had low LCAT activity as long as cholestasis could be demonstrated by the detection of LP-X. They had normal esterification rates, however, when cholestasis was no longer detectable by the very sensitive test for LP-X. Contrary to this, four patients suffering from hepatitis without cholestasis (LP-X neg.) kept their normal LCAT activities during the control period which lasted up to 18 days (Fig. 4). While LCAT activity was normal in patients with obstructive jaundice there is reasonable evidence to

Table 4. Clinical data from 11 patients with chronic liver failure

No.	Age	Sex	LCAT	LP-X	TG	Chol	CE/FC	% CE	GOT	GPT	Alk. phos.	Bilirubin	AuAg
					mg/100 ml				mU/ml		mU/ml	mg/100 ml	
1	65	♂	2.2	—	275	133	46/ 87	34	12	6	48	2.1	—
2	40	♂	2.4	—	230	220	80/140	36	40	20	42	4.8	—
3	46	♂	4.6	—	90	103	55/ 48	53	26	11	50	1.4	—
4	65	♂	2.1	—	100	110	44/ 66	40	64	22	48	2.5	+
5	66	♂	4.3	—	130	180	90/ 90	50	23	8	38	0.5	—
6	60	♂	1.7	—	229	109	14/ 95	12	18	22	25	0.5	—
7	60	♂	3.6	—	178	166	98/ 68	59	26	14	90	1.9	—
8	62	♂	2.8	+	190	138	53/ 85	38	72	88	84	14.5	—
9	31	♂	1.8	+	120	180	61/119	34	56	11	72	7.9	—
10	60	♂	1.5	+	180	240	80/160	33	20	19	80	18.5	—
11	40	♀	4.9	—	70	114	59/ 55	52	30	32	62	8.1	—

Table 5. Clinical data from patients with hepatitis and intrahepatic cholestasis (I/), hepatitis without cholestasis (II/), and extrahepatic biliary obstruction (III/) under the course of therapy

No.	Days after admission	LCAT	LP-X	TG	Chol	CE/FC	% CE	GOT	GPT	Alk. phos.	Bilirubin	AuAg
				mg/100 ml				mU/ml		mU/ml	mg/100 ml	
I/4	1	3.9	+	118	119	47/ 72	39	228	240	41	4.5	+
	12	7.0	—	258	260	170/ 90	65	13	10	28	2.8	+
I/5	1	4.6	+	204	143	44/ 99	30	564	528	78	10.8	+
	12	8.0	—	274	196	125/ 71	64	76	96	28	1.0	+
I/8	1	2.4	+	172	183	47/136	25	360	580	70	2.5	—
	4	3.7	+	200	186	56/130	30	186	360	34	2.7	—
	14	6.3	—	180	151	128/ 23	71	24	36	41	0.9	—
I/9	1	3.5	+	75	230	39/191	17	128	180	71	11.2	—
	4	3.7	+	100	205	34/171	16	840	608	48	4.6	—
	10	5.9	—	110	379	171/208	45	46	44	70	5.9	—
	17	7.6	—	105	212	156/ 56	73	27	23	57	2.3	—
I/14	1	3.1	+	200	325	69/256	21	626	528	68	6.8	—
	4	3.7	+	180	216	132/ 84	61	240	320	72	8.4	—
	17	6.7	—	150	220	145/ 75	66	40	80	39	1.9	—
II/5	1	8.4	—	80	154	88/ 66	57	160	120	68	1.0	—
	3	6.4	—	100	116	66/ 50	58	160	180	68	1.0	—
	6	9.0	—	100	150	99/ 51	66	24	24	50	0.8	—
	10	9.8	—	100	150	99/ 51	66	20	19	50	0.8	—
II/6	1	6.9	—	112	204	129/ 75	62	492	240	80	0.6	—
	5	6.4	—	120	191	125/ 66	65	360	240	60	0.5	—
	8	6.4	—	120	200	130/ 70	65	132	204	32	0.5	—
II/9	1	8.8	—	210	259	135/124	52	90	52	80	3.8	—
	17	8.0	—	210	247	149/ 88	62	88	50	82	3.8	—
II/12	1	7.7	—	200	240	156/ 84	65	24	36	71	0.9	—
	6	6.7	—	200	249	161/ 88	65	10	5	40	0.7	—
III/7	1	5.2	+	250	505	81/424	16	80	260	210	13.0	—
	12	2.3	+	300	589	73/525	12	140	280	230	18.0	—
	24	6.7	—	140	175	93/ 82	53	9	8	40	1.0	—

suggest that in the course of time LCAT activity may fall to some extent secondary to hepatocellular disturbance. Two patients with extra hepatic biliary obstruction due to gall stones were serially studied before and after operation. It was found that prior to the operation, when the obstruction had persisted for approximately two weeks, LCAT activity was slightly decreased but it reached normal values again ten days after operation.

In order to exclude possible inhibitors in the plasma of patients from group I–IV the following experiments were carried out. Normal plasma was diluted 1:1 with patients plasma (group I–IV). Enzymatic activity was always 50% of the activity

measured without dilution. Thus, at least by this method no circulating inhibitor could be detected in any group.

Discussion

In this study we determined in parallel LCAT activity and lipoprotein-X in patients with various forms of liver disease. Positive or negative immunochemical reaction for LP-X allowed us to divide patients with hepatitis into two groups: group I with and group II without cholestasis. The validity of this distinction has been demonstrated previously [14]. Patients from these groups had distinct differences in their LCAT activity. While patients with hepatitis and cholestasis were LP-X positive, they had low esterifying activity in their plasma. Their LCAT activity returned to normal levels, however, when cholestasis was no longer demonstrable by immunochemical means. Calandra *et al.* [6] has already shown that low LCAT activity in liver disease is primarily due to a reduction in the plasma concentration of this enzyme. Neither inhibitors nor a lack of an activator could be demonstrated in plasma of patients with deranged liver function. Similar conclusions were reached by Simons [2] and Gjone [3, 4]. We could also confirm this observation. The cause of low plasma LCAT activity in liver disease is not known yet. In agreement with observations by Glomset and others [7, 24, 25] we were not able to measure LCAT activity in rat liver homogenates obtained after liver perfusion with sodium chloride solution *in vivo*. Yet higher LCAT activities were found in the liver veins compared to prehepatic blood vessels. These findings are in agreement with recent observations by Simon and Boyer [23] who demonstrated a continued increase of LCAT activity in the perfusion fluid in an isolated rat liver system.

There are controversial findings in the literature with regard to the plasma concentration of LCAT activity in patients with obstructive liver disease [2–7, 26]. From our data we would like to conclude that low LCAT activity in obstructive jaundice is probably due to a secondary hepato-cellular disturbance. We always found normal enzyme activity in patients from group III when according to the patients' history and their admission to the hospital, the time interval between the onset of the disease and the LCAT determination was short. Once obstruction had lasted for some time a decrease in LCAT activity could be measured. These results would support animal experiments by Kattermann and Wolfrum [15] and Calandra *et al.* [16] who found only little change or even activation of LCAT activity in rats after administration of α-naphthyl-iso thiocyanate or after obstruction by ligation of the bile duct. In these experiments a fall in the LCAT activity occurred 21 days after the obstruction. It is of interest that serum from patients with biliary obstruction had a stimulating effect on the assay [2]. Though it is now well known that LCAT reacts preferentially with high-density lipoproteins and to a lesser extent against low-density lipoproteins, lipoprotein-X might be an unusual good substrate for LCAT. Studies to clarify this possible mechanism are currently under investigation in our laboratory.

The correlation between LCAT activity and LP-X in our patients possibly supports the hypothesis suggested by Simon and Scheig [2] that the esterifying activity in plasma corresponds to the overall severity of the liver disease. As patients with cholestasis from group I and III, who were all LP-X positive, had distinctly different LCAT concentration in their plasma, this enzymatic activity must be influenced by intra hepatic factors which are not yet understood.

From the statistical analyses which revealed a positive correlation between LCAT activity and cholesterol with a p value of only 0.05 we would like to conclude that changes in LCAT activity may only in part be responsible for the fall in the ester: free ratio in liver disease, but they cannot account for its total magnitude. Cholesterol concentration depends on two factors: on LCAT activity and on the level of an abnormal lipoprotein, LP-X, which has a high free cholesterol content.

We may speculate on the clinical importance of these studies. Parallel determinations of LCAT and LP-X may prove to be useful laboratory tests to differentiate not only between obstructive and non-obstructive jaundice, but also between extrahepatic biliary obstruction and intrahepatic cholestasis. A large clinical trial which will further support this concept is in progress [27]. Lipoprotein-X synthesis and altered LCAT activity concentration in plasma are certainly a direct consequence of liver damage. The exact metabolic pathway, however, remains to be further elucidated.

Acknowledgements. The histological diagnoses of liver biopsies were kindly performed by Prof. Dr. W. Doerr, Institute of Pathology, University of Heidelberg. We would, furthermore, like to thank Prof. Dr. H. Immich, Department of Statistics and Medical Documentation, University of Heidelberg, for performing the statistical analyses. We also thank Dr. J. Papenberg, Department of Internal Medicine, University of Heidelberg, for his help with the animal experiments. The excellent technical assistance of Miss Brigitte Walter and Miss Claudia Ruppert is very much appreciated. The study was supported by grants from the Deutsche Forschungsgemeinschaft.

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Printed in Germany