Significance of the LP-X Test in Differential Diagnosis of Jaundice[1]

D. Seidel, H. Gretz, and Claudia Ruppert

In recent years it has been well documented that the characteristic increase in plasma lipoproteins in patients with obstructive jaundice is the result of the presence of a low-density lipoprotein (relative density 1.006–1.063 g/ml) of abnormal composition and properties. This abnormal lipoprotein has been designated "LP-X." The development of a simple immunologic test system for determining LP-X provides the basis for a new clinical chemical test that is of use in the differential diagnosis of jaundice. In this study, 2680 LP-X determinations were performed on 1481 subjects: 1309 patients with or without liver disease, and 172 healthy volunteers. Statistical analysis of this series revealed a power of 0.99 and a specifity of 0.98 to demonstrate or exclude cholestasis. In this regard the new test is superior to other blood-chemical assessments. It was never positive in patients without liver disease. However, the LP-X test alone is not adequate to distinguish between intrahepatic cholestasis and extrahepatic biliary obstruction.

Additional Keyphrases: *Plasma lipoproteins • liver disease • immunochemical test to demonstrate or exclude cholestasis • diagnostic aid • alkaline phosphatase assay compared • normal values • serum aminotransferase (SGOT, SGPT) activities*

The liver is the major site of synthesis of plasma lipoproteins. Because abnormal serum lipid patterns are often associated with abnormal liver function, it is reasonable to anticipate that liver disease will result in alterations of plasma lipoproteins. The best known example is hypercholesterolemia and hyperphospholipidemia in patients with biliary obstruction or cholestasis. The increased serum cholesterol in cases of obstructive jaundice is in the form of unesterified cholesterol; thus, the percentage of serum cholesterol present in esterified form is decreased, although in absolute amounts esterified cholesterol concentrations usually remain normal as long as liver function otherwise remains good. The increment in serum phospholipid concentration is greater than that of cholesterol, resulting in a cholesterol:phospholipid ratio that is characteristically low.

Recent studies *(1–9)* have well documented that the characteristic elevation of unesterified cholesterol and phospholipids in patients with obstructive jaundice is the result of the presence of a low-density lipoprotein (LDL)[2] of abnormal composition and properties. This abnormal plasma lipoprotein has been designated "lipoprotein-X" (LP-X) *(4)*. The protein–lipid composition of the isolated LP-X is unique: 6% protein, 66% phospholipids, 22% unesterified cholesterol, 3% cholesterol esters, and 3% triglycerides *(4)*. Among bile acids present in LP-X (2–3% by wt), lithocholic acid, known to be hepatotoxic, is present in relatively large amounts. The protein moiety of LP-X consists of a combination of 40% albumin and 60% apolipoprotein C *(6)*, the third typical apolipoprotein (in addition to apo A and apo B) present as major apolipoprotein of α - and β-lipoproteins. With respect to the structure of LP-X and to the structural relations of the proteins to the lipids in this abnormal plasma lipoprotein, it has been demonstrated that LP-X (S_f value: 17) *(5)* appears as a spherical particle 30.0–70.0 nm *(7, 8)*[3] in diameter; having a strong tendency to aggregate (see Figure 1); and with a wall consisting of a phospholipid, cholesterol, and apo C. The albumin portion of LP-X seems to be masked in the core of the native particle *(6, 8)*.[3] It has been proposed *(6)*,[3] that the specific combination of apo C and albumin plays an important role in maintaining the structural integrity of this lipoprotein particle characterized by a phospholipid:protein ratio of 11. One important characteristic feature of LP-X is its electrophoretic mobility toward the

From the Medical Clinic (Ludolf-Krehl-Klinik) University of Heidelberg Medical School, Bergheimerstrasse 58, 69 Heidelberg, G.F.R.

[1]A preliminary report was presented at the 8th International Congress on Clinical Chemistry, June 1972, Copenhagen.

Received Oct. 3, 1972; accepted Nov. 9, 1972.

[2]Nonstandard abbreviations used: HDL, high-density lipoproteins, d 1.063–1.21 g/ml; LDL, low-density lipoproteins, d 1.006–1.063 g/ml, S_f 0-20; VLDL, very-low-density lipoproteins, d 1.006 g/ml, S_f >20; LP-X, abnormal lipoprotein characterizing cholestasis and obstructive jaundice; apo A, major protein portion of the α-lipoproteins; apo B, major protein portion of the β-lipoproteins; apo C, major protein portion of the VLDL; LCAT, lecithin:cholesterol acyltransferase.

[3]Seidel, D., Structure of lipoprotein-X. Eur. Atherosclerosis Group Meeting, Paris, Oct. 1970.

[Reprinted from CLINICAL CHEMISTRY, 19, 86 (1973).]

cathode on agar gel and toward the anode on agarose gel, starch gel, or paper electrophoresis *(4, 6)*. This is true for LP-X in whole plasma as well as for isolated LP-X.

After a rapid and very sensitive immunologic test system was introduced for determining LP-X in whole plasma or serum *(10)* and because preliminary screening tests *(11)*[4] indicated that the appearance of LP-X is limited to those subjects with obstructive jaundice or cholestasis (as determined by other lines of evidence), we undertook this study to evaluate the significance of the new test in the differential diagnosis of jaundice. This seemed very justified, because all known biochemical tests that are supposed to confirm or exclude cholestasis or obstructive jaundice are not specific indices of liver disease or of particular lesions and may depart from the characteristic pattern to such an extent as to be misleading in diagnosis. Many such erroneous diagnoses are recorded in the literature.

Materials and Methods

A total of 2680 LP-X determinations were made on the serum of 1481 subjects. Of these, 1309 were patients at the University Hospitals of the University of Heidelberg Medical School and at local hospitals in the Heidelberg area, and 172 were healthy voluntary donors of various ages, for whom liver disease was excluded by detailed history and determinations of the serum transaminases (EC 2.6.1.1 and 2.6.1.2) and alkaline phosphatase (EC 3.1.3.1), and in some instances by the determination of D-glutamyl transferase (EC 2.3.2.1) activity.

Our data include, without further selection, all cases presenting with an unequivocal diagnosis and, in relation to special questions, all those in whom histologic or other anatomical findings were available.

The LP-X test was performed as previously described *(10)*. The blood was usually drawn after an overnight fast, allowed to clot, and the serum separated by low-speed centrifugation. Immunoelectrophoresis was performed in a 1 g/100 ml "Bacto-Agar" gel (Difco Labs., Detroit, Mich. 48232), with use of barbital buffer (pH 8.6, 50 mmol/liter). The agar–buffer mixture was boiled for 20 min in a water bath, allowed to cool to 55°C, and 36 ml of the gel was applied to one slide frame (LKB, Bromma, Sweden) for electrophoresis carrying six glass plates, 2.5 × 8 cm. Before electrophoresis the gel plates were stored for at least 6 h in a moist chamber at room temperature. A total of six antigen wells with a diameter of 2.5 mm were punched (Figure 2) in each slide (per frame, a total of 36) and about 10 μl of the patients' sera was applied. Electrophoresis was carried out for 50 min in an LKB electrophoresis apparatus, at a constant voltage of 8 V/cm. Immediately after electrophoresis, the remaining liquid in the antigen well was removed and about 10 μl of anti-LP-X serum was applied to the gel surface at a distance of 3–5 mm toward the cathode to each antigen well. The plates were then incubated for 3–5 h at 37°C in a moist chamber, to develop the immunoprecipitin reaction. When there is an immunoprecipitin reaction to the left (toward the cathode) of the antigen well, LP-X is positive (Figure 2, *P*). If this characteristic precipitin reaction for LP-X is absent (Figure 2, *N*), the LP-X test is negative. The foggy spots to the right (toward the anode) of the antigen well are nonspecific artifacts, and therefore have no diagnostic significance. Incubation time is shorter, as compared to the conventional immunoelectrophoresis technique, because diffusion distance between antiserum and antigen is diminished to almost zero. In addition, and in contrast to conventional immunoelectrophoresis, the method described conserves antiserum and avoids an excess of antiserum, which may be important for serum with an extremely low LP-X concentration.

The specific rabbit antisera to LP-X we used were prepared in this laboratory as described previously *(4)*, but antiserum to LP-X is now commercially

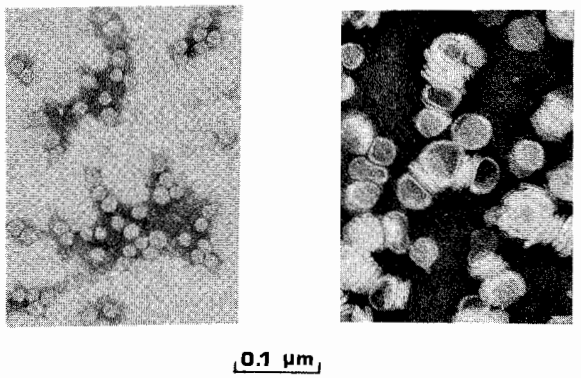

Fig. 1. Size and shape of LP-X in comparison with normal β-lipoprotein (B. Agostini and D. Seidel, Caratterizzazione, struttura ed importanza diagnostica della lipoproteina plasmatica abnorme—LP-X—di pazienti con ittero colostatico, *Fegato*, in press)
(Right) LP-X, 30.0–70.0 nm; (left) β-lipoprotein, 20.0–25.0 nm (150,000X)

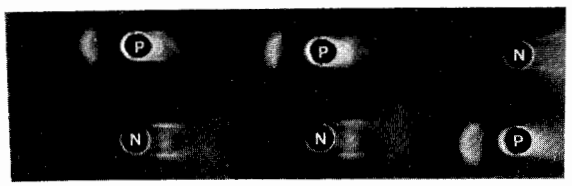

Fig. 2. Immunoelectrophoretic patterns of whole serum on 1% agar gel
P is LP-X positive sera of different patients with obstructive jaundice or cholestasis; *N* is LP-X negative sera from various patients without cholestasis. The rabbit anti-LP-X serum was applied on the gel surface after running the electrophoresis

[4]Poley, J. R., Alaupovic, P., Seidel, D., and McConathy, W. J., Differential diagnosis between "neonatal hepatitis" and extrahepatic biliary atresia in infants. 4th World Congress of Gastroenterology, Copenhagen, Denmark, 1970.

available from Immuno Diagnostics, Industriestrasse, Vienna, Austria. The aminotransferases and alkaline phosphatase were measured kinetically with the LKB 8600 Enzyme-Analyzer, and bilirubin with the AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N.Y. 10591).

Results

Table 1 summarizes the results of the LP-X test in the differential diagnosis of jaundice. The patients are divided into three groups: those with anatomically proven biliary obstruction or cholestasis, those without cholestasis excluded by histologic examination, and those without histologic examination or with an uncertain histologic report. Although the result of the LP-X test was confirmed in most cases, alkaline phosphatase activity was not abnormally great in 11% of patients with cholestasis, and distinctly supranormal (>100 U/liter; upper normal range: 48 U/liter) in 16% of the patients without cholestasis as determined by anatomic lines of evidence.

LP-X was positive in all 151 patients with extrahepatic biliary obstruction (Table 2). The nine patients with biliary atresia were infants, one to six months old. It is noteworthy that the alkaline phosphatase activity in this group with extrahepatic obstruction was normal in 10% of cases and <100 U/liter in 23% of cases. Alanine aminotransferase activity in this group of patients was increased to >50 U/liter (normal, up to 12 U/liter) in 48% of cases. Ten to twelve days after successful operative relief of biliary obstruction, we generally could no longer demonstrate LP-X.

Histologic confirmation of the diagnosis was available in 90 of 166 cases with cirrhosis of the liver (Table 2); the remaining 76 had clinically unequivocal cirrhosis. In the group with histologically proven cirrhosis, the LP-X test was positive in 28 of 30 cases with intrahepatic stasis, and was always negative when intrahepatic biliary stasis was excluded.

Histologic findings were available in 220 of 380 cases with unequivocal hepatitis (Table 2). The LP-X test was positive in 5 of 101 cases in whom cholestasis was excluded histologically. In all of these LP-X positive cases, a diagnosis of biliary stasis was excluded by blind (needle) liver biopsy. On the other hand, the LP-X test was positive in 58 of 59 cases of hepatitis with histologically proven cholestasis. Altogether, 26% of the patients in the hepatitis group were LP-X positive. The incidence of cholestasis associated with hepatitis ranges from 5% to more than 50% *(12–14)*, the percentages appearing to vary in different epidemics. Therefore, the figure of 26% presumably is representative of our own series only. It is nevertheless remarkable that 65% of our patients with hepatitis had supranormal alkaline phosphatase activities.

In patients without liver disease the LP-X test was invariably negative (Table 2). 90% of the patients with hemolytic jaundice were newborns. The alkaline phosphatase activity was elevated in 35% of these patients without liver disease. If one subtracts the 172 healthy volunteers, every second subject in this group had an abnormally high alkaline phosphatase activity.

Statistical analysis (fourfold χ^2 test) for the significance of the LP-X test to demonstrate or exclude cholestasis was performed on the group on whom the diagnosis was confirmed by anatomic lines of evidence. It revealed a power of 0.99 and a specifity of 0.98. No other conventional blood chemical test was found to be as sensitive or specific. As examples, results of the analysis of variance are presented on ran-

Table 1. LP-X Test in the Differential Diagnosis of Jaundice Resulting from Liver Dysfunction

Diagnosis	n	LP-X +	LP-X −	Alk. phosphatase
Cholestasis				
Extrahepatic biliary obstruction	151	151	0	not elevated (<45 U/liter) in 11%
Hepatitis with cholestasis	59	58	1	
Cirrhosis with cholestasis	30	28	2	
Tumor of the liver	26	26	0	moderate elevation (45–100 U/liter) in 25%
Fatty liver with cholestasis	3	3	0	
Drug-induced liver dysfunction with cholestasis	3	3	0	distinctly elevated (>100 U/liter) in 64%
Other forms of intrahepatic cholestasis	8	8	0	
Totals	280	277	3	
No cholestasis				
Hepatitis without cholestasis	161	5	156	not elevated (<45 U/liter) in 45%
Cirrhosis without cholestasis	60	0	60	
Fatty liver without cholestasis	41	2	39	moderate elevation (45–100 U/liter) in 39%
Drug-induced liver dysfunction without cholestasis	5	0	5	
Other forms of liver dysfunction without cholestasis	10	2	8	distinctly elevated (>100 U/liter) in 16%
Totals	277	9	269	
No histologic data				
Hepatitis	148	81	67	
Cirrhosis	76	28	48	
Others	26	7	19	
Histologic results uncertain	12	3	9	

Table 2. Results of LP-X Test in Normal Persons and in Patients with Liver or Other Disease

Extrahepatic Biliary Obstruction

Diagnosis	n	LP-X −	LP-X +
Stone	99	99	0
Tumor	38	38	0
Stenosis of the bile duct	5	5	0
Atresia of the bile duct	9	9	0
	151	151	0

Cirrhosis of the Liver

Diagnosis	n	LP-X +	LP-X −
Prim. biliary cirrhosis	16	15	1
Cirrhosis with cholestasis	14	13	1
Cirrhosis without cholestasis	60	0	60
Subtotals	90	28	62
No histology	76	28	48
Totals	166	56	110

Hepatitis

Diagnosis	n	LP-X +	LP-X −
Acute hepatitis without cholestasis	67	3	64
Chronic hepatitis without cholestasis	94	2	92
Acute hepatitis with cholestasis	38	37	1
Chronic hepatitis with cholestasis	21	21	0
Subtotals	220	63	157
Histology uncertain	12	3	9
No histology	148	81	67
Totals	380	147	233

Patients without Liver Disease

Diagnosis	n	LP-X +	LP-X −
Hemolytic anemia	94	0	94
Heart diseases	85	0	85
Metabolic diseases	81	0	81
Gastrointestinal dis.	32	0	32
Lung diseases	28	0	28
Kidney diseases	13	0	13
Infectious diseases	33	0	33
Skeletal diseases	9	0	9
Others	115	0	115
Healthy controls	172	0	172
	662	0	662

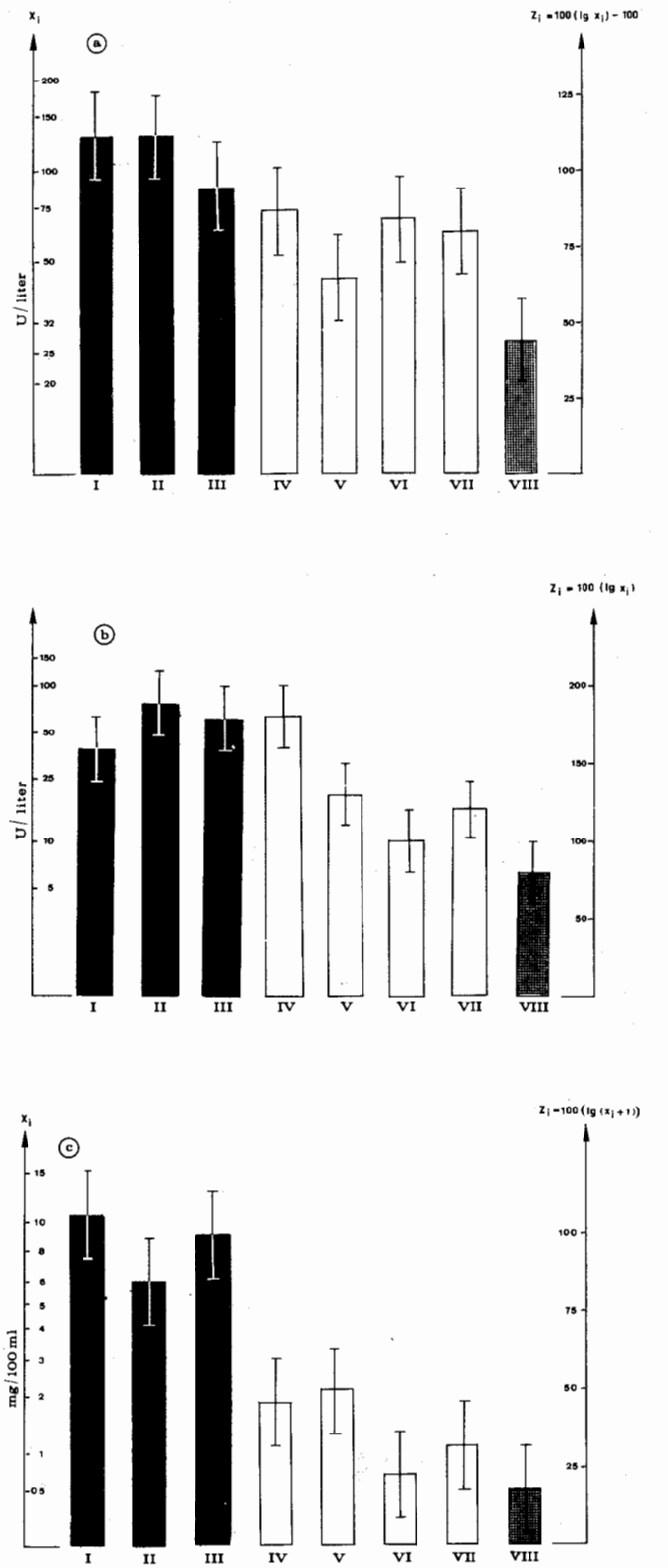

Fig. 3 (*a–c*). Results of a one way analysis of variance (model I; according to R. A. Fisher: Statistical methods for research workers, Oliver and Boyd Ltd., Edinburgh, 1958) on randomized groups (n = 17; confidence interval, 0.95). All determinations on these patients, including the determination of LP-X, were performed on the same day

I = extrahepatic biliary obstruction
II = hepatitis with cholestasis
III = cirrhosis with cholestasis
IV = hepatitis without cholestasis
V = cirrhosis without cholestasis
VI = patients without liver disease
VII = heart patients
VIII = healthy controls
a = alkaline phosphatase
b = alanine aminotransferase
c = bilirubin

domized groups of 17 subjects for alkaline phosphatase (Figure 3a), for alanine transaminase (Figure 3b), and for bilirubin (Figure 3c) with a confidence interval of $\alpha = 0.95$. The overlap of the confidence intervals shows that it is not possible to distinguish between cholestasis and noncholestasis by using one of these assays as the criterion.

Table 3 includes all cases in which the diagnosis was unequivocal on the basis of a histologic or other anatomic evidence and in which the results of the

Table 3. Divergent Results of the Alkaline Phosphatase Determination and the LP-X Test

A. LP-X test negative—alk. phos. activity distinctly supranormal (> 100 U/liter)

Diagnosis	No. patients
Hepatitis without cholestasis	14
Cirrhosis without cholestasis	13
Fatty liver without cholestasis	10
Other forms of liver dysfunction without cholestasis	7
Patients without liver disease	52
Total	96

B. LP-X test positive—alk. phos. activity not supranormal (< 48 U/liter)

Diagnosis	No. patients
Extrahepatic biliary obstruction	9
Hepatitis with cholestasis	8
Chronic hepatitis without cholestasis	1
Cirrhosis with cholestasis	3
Drug-induced intrahepatic biliary obstruction	3
Other forms of intrahepatic biliary obstruction	7
Total	31

LP-X test and the alkaline phosphatase test were discordant. This comparison illustrates the clinical value of the LP-X test in cases in which alkaline phosphatase activity alone could have led to an erroneous diagnosis.

Discussion

Because of improved techniques for isolating and characterizing plasma lipoproteins, efforts have been made to define more precisely the nature of serum lipid disturbance in liver disease.

Besides alterations in the serum HDL and VLDL (15) in severe liver disease, one characteristic feature of lipoprotein disturbance is the formation of an abnormal LDL, designated LP-X, in patients with cholestasis or extrahepatic biliary obstruction *(1–11)*.[3]

The unique physicochemical properties of LP-X that cause its abnormal electrophoretic mobility on agar-gel made possible a new immunologic test system for its easy determination.

Because LP-X was never found in healthy subjects and because there was no biochemical parameter available to demonstrate or exclude cholestasis specifically, efforts to prove the clinical relevance of this new test in the differential diagnosis of jaundice were justified.

Our results strongly indicate a high power and specifity of this test in demonstrating or excluding cholestasis. In this regard, the new test is superior to all known blood chemical tests. Note that the LP-X test was positive in all cases with extrahepatic biliary obstruction, even though some cases had normal alkaline phosphatase activities. Conversely, negative results for the LP-X test in association with a distinctly increased alkaline-phosphatase are also of great clinical value. None of 96 subjects with these findings had histologic or other anatomic evidence of cholestasis. An operation thus seems to be indicated only when the LP-X test is positive. However, the test will not distinguish between intrahepatic cholestasis and extrahepatic biliary obstruction. This was not to be expected, because the most likely cause of LP-X in the plasma of patients with obstructive jaundice is a complicated secondary metabolic disturbance, which probably is independent of the actual site of biliary obstruction but on which the liver plays a central role. The precise nature of the factors responsible for the formation of lipoprotein-X is still unknown.

Of particular interest in this regard are results on the characterization of the plasma lipoproteins in patients with familial LCAT deficiency. Patients suffering this rare metabolic disease also show lipoproteins very similar to LP-X *(16)*[5], although no obvious signs of liver dysfunction are apparent in these patients. Because patients with various forms of liver disease also show decreased concentrations of the LCAT enzyme *(17–22)*, several disturbances seem to be responsible for the described alterations of lipid metabolism. However, the possibility that patients with familial LCAT deficiency also suffer some sort of liver dysfunction on a molecular (enzymatic) level will have to be carefully excluded. From a clinical chemical point of view, it is of interest that a combined determination of the herein described LP-X test and the determination of the LCAT enzyme in patients with liver disease indicated the possibility of differentiating not only between obstructive and nonobstructive jaundice, but also between extrahepatic biliary obstruction and intrahepatic cholestasis *(22)*. This correlation does not hold for the LCAT determination in combination with any other blood chemical determination.

The statistical analysis was kindly performed by Professor Dr. H. Immich, Dept. of Statistics and Medical Documentation, University of Heidelberg. This work was supported by grants from the Deutsche Forschungsgemeinschaft.

References

1. Eder, H. A., Russ, E. M., Pritchett, R. A. R., Wilber, M. M., and Barr, D. P., Protein-lipid relationships in human plasma: In biliary cirrhosis, obstructive jaundice, and acute hepatitis. *J. Clin. Invest.* **34,** 1147 (1955).

2. Russ, E. M., Raymunt, J., and Barr, D. P., Lipoproteins in primary biliary cirrhosis. *J. Clin. Invest.* **35,** 133 (1956).

3. Switzer, S., Plasma lipoproteins in liver disease. I. Immunologically distinct low-density lipoproteins in patients with biliary obstruction. *J. Clin. Invest.* **46,** 1855 (1967).

[5]Seidel, D., Gjone, E., Blomhoff, J. P., and Geisen, H. P., Plasma lipoproteins in patients with familial plasma lecithin:cholesterol acyltransferase deficiency: Apolipoprotein composition of isolated fractions. Int. symposium on lipid metabolism, obesity, and diabetes mellitus: Impact upon atherosclerosis, Wiesbaden-Oberursel, Germany, April 1972.

4. Seidel, D., Alaupovic, P., and Furman, R. H., A lipoprotein characterizing obstructive jaundice. I. Method for quantitative separation and identification of lipoproteins in jaundiced subjects. *J. Clin. Invest.* **48,** 1211 (1969).

5. Mills, G. L., Seidel, D., and Alaupovic, P., Ultracentrifugal characterization of a lipoprotein occurring in obstructive jaundice. *Clin. Chim. Acta* **20,** 239 (1969).

6. Seidel, D., Alaupovic, P., Furman, R. H., and McConathy, W. J., A lipoprotein characterizing obstructive jaundice. II. Isolation and partial characterization of the protein moieties of low density lipoproteins. *J. Clin. Invest.* **49,** 2396 (1970).

7. Hamilton, R. L., Havel, R. J., Kane, J. P., Blaurock, A. E., and Sata, T., Cholestasis: Lamellar structure of the abnormal human serum lipoprotein. *Science* **172,** 475 (1971).

8. Seidel, D., Agostini, B., and Müller, P., Structure of an abnormal plasma lipoprotein (LP-X) characterizing obstructive jaundice. *Biochim. Biophys. Acta* **260,** 146 (1972).

9. Picard, J., Veissiere, D., Voyer, F., and Bereziat, G., Composition en acides gras des phospholipides dans les lipoproteines seriques anomales de la cholestase. *Clin. Chim. Acta* **36,** 247 (1972).

10. Seidel, D., A new immunochemical technique for a rapid, semiquantitative determination of the abnormal lipoprotein (LP-X) characterizing cholestasis. *Clin. Chim. Acta* **31,** 225 (1971).

11. Seidel, D., Schmitt, E. A., and Alaupovic, P., An abnormal low density lipoprotein in obstructive jaundice II: Significance in the differential diagnosis of jaundice. *Deut. Med. Wochenschr.* **15,** 671 (1970).

12. Dubin, J. N., and Johnson, F. B., Chronic idiopathic jaundice with unidentified pigment in liver cells. A new clinicopathologic entity with report of 12 cases. *Medicine* (Baltimore) **33,** 155 (1954).

13. Smetana, H. F., Hepatitis frontiers. *Henry Ford Hosp. Int. Symp.* **77,** (1957).

14. Wildhirt, E., Cholostatischer Ikterus. *Ergeb. Inn. Med. Kinderheilk.* **24,** 80 (1966).

15. Seidel, D., Greten, H., Geisen, H. P., Wengeler, H., and Wieland, H., Further aspects on the characterization of high and very low density lipoproteins in patients with liver disease. *Eur. J. Clin. Invest.* **2,** 359 (1972).

16. Torsvic, H. Berg., Magnani, H. N., McConathy, W. J., and Alaupovic, P., Identification of the abnormal cholestatic lipoprotein (LP-X) in familial lecithin: Cholesterol deficiency. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **42,** 165 (1972).

17. Turner, K. B., McCormack. G. H., and Richards, A., The cholesterol-esterifying enzyme of human serum. I. In liver disease. *J. Clin. Invest.* **32,** 801 (1953).

18. Simon, J. B., and Scheig, R., Serum cholesterol esterification in liver disease, importance of lecithin-cholesterol acyltransferase. *New Engl. J. Med.* **283,** 841 (1970).

19. Gjone, E., and Blomhoff, J. P., Plasma lecithin-cholesterol acyltransferase in obstructive jaundice. *Scand. J. Gastroenterol.* **5,** 305 (1970;.

20. Gjone, E., Blomhoff, J. P., and Wiencke, I., Plasma lecithin: cholesterol acyltransferase activity in acute hepatitis. *Scand. J. Gastroenterol.* **6,** 161 (1971).

21. Calandra, S., Martin, M. J., and McIntyre, N., Plasma lecithin cholesterol acyltransferase in liver disease. *Eur. J. Clin. Invest.* **1,** 352 (1971).

22. Wengeler, H., Greten, H., and Seidel, D., Serum cholesterol esterification in liver disease. Combined determinations of lecithin-cholesterol acyltransferase and lipoprotein-X. *Eur. J. Clin. Invest.* **1,** 372 (1971).