Investigation of the Abnormal Low-Density Lipoproteins Occurring in Patients with Obstructive Jaundice

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The abnormal lipoproteins of the density range 1.019–1.063 g/cm³ occurring in the plasma of patients with obstructive jaundice were studied. Subfractionation of this density class by combined sodium phosphotungstate precipitation, ultracentrifugation, and column chromatography on hydroxyapatite and agarose gel yielded essentially three fractions: (1) lipoprotein-X, (2) a triglyceride-rich lipoprotein for which we propose the term lipoprotein-Y and (3) an abnormal lipoprotein, lipoprotein-B. Marked differences between these fractions with respect to electron-microscopic appearance, hydrated densities, chemical composition and immunochemical characteristics were observed. The protein moiety of lipoprotein-X consisted primarily of apolipoprotein-C and albumin. Lipoprotein-Y showed, in addition to apolipoprotein-C, the presence of apolipoprotein-B. The 'lipoprotein-B' fraction isolated from sera of these patients had higher triglyceride and free cholesterol contents than that of normal individuals and an unusually high content of apolipoprotein-C. The relative distribution of lipoprotein-X, lipoprotein-Y and 'lipoprotein-B' varied from patient to patient. The importance of considering the existence of lipoprotein-Y in screening patients for cholestasis by the lipoprotein-X test is discussed.

Kunkel & Slater (1952) first described the occurrence of abnormal lipoproteins in the serum of patients with liver diseases. These lipoproteins have been investigated in the past by several groups (Switzer & Satenstein, 1967; Burstein & Caroli, 1968; Seidel *et al.*, 1969; Picard & Veissiere, 1970). Since then the general interest was directed to a lipoprotein called lipoprotein-X, which was believed to be specific for cholestasis (Seidel *et al.*, 1973). However, this abnormal lipoprotein was later also identified in the serum of patients with familial phosphatidylcholine-cholesterol acyltransferase deficiency (Torsvik *et al.*, 1972; Utermann *et al.*, 1972), a metabolic disorder normally not correlated with cholestasis. Clinical surveys of patients with liver diseases (Vergani *et al.*, 1973; Ober & Witt, 1975) have cast further doubt on the unequivocal cholestasis specificity of lipoprotein-X.

The characteristic changes in lipoprotein patterns of several density classes have been investigated

(Seidel *et al.*, 1972; Blomhoff, 1974). Striking similarities exist between the abnormal lipoproteins observed in patients suffering from obstructive liver diseases and those with familial phosphatidylcholine-cholesterol acyltransferase deficiency (Norum *et al.*, 1971; Utermann *et al.*, 1975). The subfractionation of LD-lipoproteins† of transferase-deficient patients revealed the presence of a minimum of three fractions differing in size and flotation coefficients (Glomset *et al.*, 1975). One of these fractions exhibited an unusually high content of triglycerides (Norum *et al.*, 1971). A triglyceride-rich lipoprotein differing from lipoprotein-B and lipoprotein-X was also noticed in the LD-lipoprotein fraction of cholestatic patients, and its isolation and partial characterization have been described (Müller *et al.*, 1974). This lipoprotein, so far called β_2 -LP, a term which fails to demonstrate its nature as a lipoprotein in diseased states, will be called lipoprotein-Y in the present paper. The isolation and characterization of lipoprotein-Y among other abnormal lipoproteins

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‡ Abbreviations: LD-lipoproteins, low-density lipoproteins; VLD-lipoproteins, very-low-density lipoproteins; HD-lipoproteins, high-density lipoproteins.

occurring in the LD-lipoprotein fraction of patients with obstructive jaundice is the subject of the present paper.

Materials and Methods

Blood samples

Blood samples for the preparation of abnormal lipoproteins were obtained primarily from the surgery department of the University Hospital from patients suffering from extrahepatic biliary obstruction caused by carcinomas of the head of the pancreas, or gallstones. In some cases strongly lipoprotein-X-positive sera were used of patients with liver diseases that had not been further identified. The subjects have given informed consent to the taking of blood samples. The presence of lipoprotein-X and especially of lipoprotein-Y was tested by the immunochemical method proposed by Seidel *et al.* (1969) and described in detail in earlier papers (Petek *et al.*, 1973; Kostner *et al.*, 1974a). Antisera used during these studies were the same as described in the earlier papers and had the same specificity as the commercially available product from Behring Werke A.G., Marburg, Germany.

Purification of the abnormal lipoproteins

The citrate/plasma or serum from lipoprotein-X-positive patients was separated from blood cells in a laboratory centrifuge immediately after sampling and blood clotting. The total VLD-lipoprotein and LD-lipoprotein fraction was first precipitated with sodium phosphotungstate as described by Burstein & Morfin (1969) as outlined earlier (Kostner *et al.*, 1974b). The precipitate was dissolved in 20 vol. of 0.1 M-NaCl, pH 8.2 (buffered by adding 0.01 vol. of 1 M-Tris/HCl), and dialysed against a 2.8% (w/w) NaCl solution (pH 8.2) of density 1.019 g/cm³. Samples of volume less than 100 ml were dialysed directly against NaCl ($\rho = 1.019$), avoiding the precipitation step. This fraction was centrifuged at 120000g for 20 h at 16°C in the 50Ti fixed-angle rotor (r_{av} , 5.9 cm) of a Beckman L-II 65 B ultracentrifuge. The upper third of each tube containing lipoproteins of $\rho < 1.019$ g/cm³ was removed by tube slicing and not further characterized during this study. The density of the lower layer was adjusted to $\rho = 1.065$ g/cm³ by adding solid NaCl and centrifuged at 122000g for 24 h at 16°C. The floating LD-lipoproteins were washed under identical conditions and dialysed exhaustively against 0.1 M-potassium phosphate buffer, pH 6.8. The buffer was prepared by a tenfold dilution of 1 M-potassium phosphate buffer, pH 6.8; this 1 M buffer was prepared by mixing 1 mol of H₃PO₄ with approx. 500 ml of water, adding 4 M-KOH to give pH 6.8, and diluting to 1 litre with water. Portions (10–15 ml) of the LD-lipoprotein fraction, containing 20–30 mg of lipoproteins/ml, were passed over a column (1.2 cm ×

15 cm) packed with hydroxyapatite (Bio-Gel HT; Bio-Rad Laboratories, Richmond, CA, U.S.A.). Stepwise elution of lipoproteins was performed with 0.1 M-, 0.25 M- and 0.65 M-potassium phosphate buffer (70 ml each), pH 6.8, prepared by dilution of the 1 M-potassium phosphate buffer. In some cases elution with a linear gradient of 0.1–0.65 M-potassium phosphate was applied. Eluted lipoproteins were collected with an LKB fraction collector equipped with an Uvicord III u.v. detector; fractions eluted as single peaks as monitored at 278 nm were combined, concentrated by dialysis against polyethylene glycol and re-chromatographed over a column (2.0 cm × 100 cm) packed with Bio-Gel A-50m (Bio-Rad), by using 0.1 M-potassium phosphate buffer, pH 6.8, as eluent. The eluted fractions were detected and collected as described for the hydroxyapatite experiment.

Intact and totally delipidated lipoproteins were investigated by immunochemical methods and by polyacrylamide-gel electrophoresis. For total delipidation, lipoproteins were dialysed against water, freeze-dried and extracted with ethanol/ether (3:1, v/v) and ether at 4°C (Kostner & Alaupovic, 1972). Lipoproteins and apolipoproteins were characterized by immunoelectrophoresis and immunodiffusion in 1% agarose gels by using monospecific antisera against apolipoproteins apoAI, apoAII, apoAIII, apoCI, apoCII, apoB and 'arginine-rich' polypeptide (Kostner & Holasek, 1972; Kostner *et al.*, 1974a).

Polyacrylamide-gel electrophoresis was performed in 10% (w/v) gels containing 8 M-urea (Kostner & Alaupovic, 1972). Chemical analysis of proteins and individual lipids were carried out by conventional procedures described earlier (Kostner & Holasek, 1972; Kostner & Alaupovic, 1972). For electron microscopy of purified lipoproteins, samples of each solution were mixed with equal volumes of 2% (w/v) sodium phosphotungstate at pH 6.8 for different lengths of time, applied on a hydrophilized carbon-coated grid and investigated in a Philips EM 300 electron microscope (Jakopitsch & Horn, 1962).

The determination of the partial specific volumes ($\bar{v}$) of different lipoprotein fractions was carried out with a precision density meter (A. Paar, Graz, Austria) as described earlier (Kratky *et al.*, 1973). Analytical ultracentrifugation was carried out with a Beckman Model E instrument. Flotation coefficients were calculated as described earlier (Kostner, 1972).

Results

As pointed out and illustrated previously (Petek *et al.*, 1973), the migration rate of lipoprotein-X towards the cathode and the shape of the precipitin lines vary from patient to patient. In some cases when

antisera specific for lipoprotein-X were used, the whole precipitin line is situated at the cathodal part of the slide or starts just at the point of application, whereas in other cases the material seems to remain at the start or even migrates to the anode. Prolonged running times lead to essentially the same results.

Fig. 1 shows the elution pattern from the hydroxyapatite column of the LD-lipoprotein fraction ($\rho = 1.019\text{--}1.063$) from a patient with obstructive jaundice. The relative heights of peaks I, II and III varied considerably from patient to patient. In some cases peak II was virtually absent, whereas in others, minute amounts of material eluted as peak I were observed. The described buffers eluted more than 95% of the applied LD-lipoproteins. On careful concentration of the eluted peaks by dialysis against polyethylene glycol at 4°C, the material stayed clear or became only slightly turbid up to concentrations of 50 mg/ml. Improper treatment of samples resulted in cloudy solutions and formation of precipitates that were impossible to solubilize in conventional buffers. For further purification, the lipoprotein fractions were chromatographed on columns of Bio-Gel A-50m. The major part of the material from clear samples of peaks I, II and III from the hydroxyapatite column was eluted as single, more-or-less symmetrical peaks with different elution volumes; small amounts of lipoproteins were eluted at the void volume and a small fraction with an elution volume characteristic of HD-lipoproteins (Fig. 2). The amount of the cloudy material eluted with the void volume (peak 1) as well as peak 3 increased considerably unless special care was taken in handling the samples. We therefore

Fig. 1. Elution pattern from hydroxyapatite of the low-density-lipoprotein fraction ($\rho = 1.019\text{--}1.063$) from a patient with cholestasis

The material from 200 ml of serum was applied and stepwise elution was performed with 0.1 M-, 0.25 M- and 0.65 M-potassium phosphate buffer, pH 6.8.

considered this latter material to be degradation products, and no further characterization was attempted. The main peaks were investigated by electron microscopy. As shown in Plate 1, all three fractions differed in size and shape. The Bio-Gel A-50m fraction 2 from material of peak I in Fig. 2 (I-2) exhibited disc-like structure with a long axis of 50 nm and a short one of 9 nm, forming stacks which are characteristic of the electron-microscopic appearance of lipoprotein-X (Forte *et al.*, 1974; Hamilton *et al.*, 1971). The Bio-Gel A 50m fraction 2 of the material eluting as peak II in Fig. 1 (II-2, lipoprotein-Y) consisted of relatively uniform spherical particles of 40 nm diameter. Stacked discs were seen only infrequently in some

Fig. 2. Separation on Bio-Gel A-50m of the material eluted from the hydroxyapatite column

A 2.0 cm $\times$ 100 cm column of Bio-Gel A-50m was eluted with 0.1 M-potassium phosphate buffer, pH 6.8; 80–150 mg of material was applied. —, Lipoprotein-X (peak I of Fig. 1); ---, lipoprotein-Y (peak II of Fig. 1); ·····, lipoprotein-B_c (peak III of Fig. 1).

lipoprotein-Y preparations. Lipoproteins eluted as Bio-Gel A-50m fraction 2 if material of peak III from hydroxyapatite experiment was applied to the column (fraction III-2, lipoprotein-B_e) exhibited a spherical shape as observed with lipoprotein-B from normal individuals with diameters of 18–20 nm. Lipoprotein-B_e was the only fraction present in the LD-lipoprotein class of these patients, except for lipoprotein-X and lipoprotein-Y, and no 'normal' lipoprotein-B has been detected. The chemical composition of the three lipoprotein entities separated from LD-lipoproteins of two different patients is shown in Table 1. The values for lipoprotein-X agree well with those published earlier (Seidel *et al.*, 1969; Picard & Veissiere, 1970; Utermann *et al.*, 1972). The composition of lipoprotein-B_e from patients with liver disease (fraction III-2), unlike lipoprotein-B from normal patients, showed increased contents of triglycerides and of free cholesterol. Characteristic of lipoprotein-Y was the high content of triglycerides, similar to some LD-lipoprotein fractions of patients with phosphatidylcholine-cholesterol acyltransferase deficiency (Norum *et al.*, 1971) or others with different forms of liver disease (Müller *et al.*, 1974).

Results of the immunochemical examination of intact lipoproteins with various kinds of antibodies are listed in Table 2. The antigen concentration in these experiments ranged from 10 to 15 mg/ml. Table 2 also contains the results of the reaction with apolipoproteins; in these experiments the concentration of antigens ranged from 2 to 15 mg/ml. The intact lipoprotein-X reacts strongly with antibodies to lipoprotein-X, apolipoprotein-CI, and weakly with antibodies to apolipoprotein-AIII; after delipidation, additional reactions with anti-albumin, immunoglobulin G, apolipoprotein-CII and apolipoprotein-CIII are observable. Lipoprotein-Y shows a similar immunochemical behaviour, except that the intact lipoproteins give a strong reaction with anti-(lipo-

protein-B). Apolipoprotein-Y reacts also with anti-(lipoprotein-B) if solubilized in detergents such as sodium dodecyl sulphate.

Some experimental proof that all the antigens are situated on the same particle can be gained from immunoelectrophoretic experiments. Plate 2 shows the immunoelectrophoretic pattern of lipoprotein-Y in 1% agarose obtained by using combined antibodies to lipoprotein-B and lipoprotein-X. Exactly the same results are obtained with anti-(lipoprotein-B) in the one and anti-(apolipoprotein-CI) in the other antiserum trough. With anti-(apolipoprotein-AIII), however, the reaction is much weaker. In pure agarose, lipoprotein-Y migrates in the position of β -globulins, whereas the admixture of increasing amounts of agar to the agarose leads to a gradual decrease of the migration rate relative to lipoprotein-B.

The material that was eluted as peak III-2, representing lipoproteins very similar to lipoprotein-B of normal sera, in all cases reacted with antibodies to lipoprotein-X, apolipoprotein-CI, -CII and -CIII, but much more weakly than the corresponding II-2 peak, lipoprotein-Y. Under identical conditions lipoprotein-B from normal sera gave no or only minute reactions with those antisera. The investigation of apolipoprotein-X, apolipoprotein-Y and apolipoprotein-B_e by polyacrylamide-gel electrophoresis in 10% gels containing 8 M-urea confirmed the result obtained by immunochemistry. Plate 3 shows the patterns of the different delipidated abnormal lipoproteins of the LD-lipoprotein class from a patient with extrahepatic cholestasis. Because of the high monomer concentration of the gel, high-molecular-weight proteins such as immunoglobulin G or apolipoprotein-B do not enter the separating gel.

Although all three abnormal lipoproteins were found in the LD-lipoprotein class ($\rho = 1.019$ –

Table 1. Chemical composition of the abnormal low-density lipoproteins in cholestasis

Values represent means of three determinations on the same preparation. Patients L. C. and M. F. suffered from cancer of the head of the pancreas, and patient A. L. from cholestasis caused by gall stones.

Lipoproteins ...		Content [% (w/w) of total lipoprotein mass]									
		Lipoprotein-X			Lipoprotein-Y			Lipoprotein-B _e			Lipoprotein-B
		L. C.	M. F.	A. L.	L. C.	M. F.	A. L.	L. C.	M. F.	A. L.	Normal subjects*
Protein	...	5.5	4.8	6.8	25.9	29.6	27.4	23.4	24.0	22.8	21.3
Phospholipids	...	59.8	61.4	63.1	23.8	16.1	18.4	25.8	24.1	26.0	22.4
Free cholesterol	...	25.0	27.9	25.5	7.6	9.7	8.8	11.2	10.2	13.4	9.5
Cholesterol ester	...	6.5	3.1	2.8	11.1	9.8	12.5	29.8	34.6	32.6	42.7
Triglycerides	...	3.2	2.8	2.5	31.6	34.8	32.9	9.8	7.1	5.2	4.1

* Values of a lipoprotein-B preparation from the pooled serum of eight normal individuals.

EXPLANATION OF PLATE I

Electron micrographs of the abnormal lipoproteins from patients with cholestasis

The bars indicate 50nm. (a) Fraction I-2 (lipoprotein-X); (b) fraction II-2 (lipoprotein-Y): the insert shows a lipoprotein-Y preparation from another individual where a larger than usual amount of stacked discs were observed; (c) fraction III-2 (lipoprotein-B_c). In all cases negative staining with 2% (w/v) sodium phosphotungstate was performed.

EXPLANATION OF PLATE 2

Immunoelectrophoretic pattern of lipoprotein-Y in 1% agar gels

The wells contain material from peak II-2 (lipoprotein-Y). The troughs were filled with anti-(lipoprotein-X), anti-(lipoprotein-B), anti-(lipoprotein-CI), and anti-(lipoprotein-AIII).

EXPLANATION OF PLATE 3

Polyacrylamide-gel electrophoresis of totally delipidated abnormal proteins from cholestasis patients

Electrophoresis was in 10% gels containing 8 M-urea. Staining was performed with Coomassie Blue. (a) Apolipoprotein-B_c; (b) apolipoprotein-X; (c) apolipoprotein-Y; (d, line drawing), normal apolipoprotein-B.

EXPLANATION OF PLATE 4

Flotation of the abnormal low-density lipoproteins isolated from patient A. L. (Table 1)

Analytical ultracentrifugation was at 52000 rev./min and 21.5°C. The density of the solvent was 1.063 g/cm³. Photographs were taken 30 min after reaching full speed. Bar angles from top to bottom: 75°, 63° and 63° respectively. (a) Lipoprotein-B_c; (b) lipoprotein-X; (c) lipoprotein-Y.

Table 2. *Immunochemical reaction of the abnormal LD-lipoproteins and their lipid-free protein moieties*

The antisera were the same as cited in Kostner *et al.* (1974a). Symbols for reaction are: +++, strong; ++, medium; +, weak; $\pm$, occasionally positive; – negative.

Antisera to:	Samples tested ...	Lipo-protein-X	Apolipo-protein-X	Lipo-protein-Y	Apolipo-protein-Y	Lipo-protein-B _e	Apolipo-protein-B _e	Lipo-protein-B (normal)	Apolipo-protein-B (normal)
Apolipoprotein-AI	–	–	–	–	–	–	–	–	–
Apolipoprotein-AII	–	$\pm$	–	$\pm$	–	–	–	–	–
Apolipoprotein-AIII	+	++	+	+	–	$\pm$	–	–	–
Apolipoprotein-CI	+++	+++	++	++	+	+	–	–	$\pm$
Apolipoprotein-CII	–	+++	–	++	+	+	–	–	–
Apolipoprotein-CIII	–	+++	–	++	+	+	–	–	$\pm$
Arginine-rich polypeptide	–	–	–	–	–	$\pm$	–	–	–
Apolipoprotein-B	–	–	++	*	+++	*	+++	–	*
Albumin	–	+	–	$\pm$	–	$\pm$	–	–	–
Immunoglobulin-G	–	+	–	?	–	–	–	–	–
Immunoglobulin-A	–	$\pm$	–	–	–	–	–	–	–

* Positive if solubilized in sodium dodecyl sulphate containing buffers.

Table 3. *Flotation coefficients and partial specific volumes of the 'pathological' LD-lipoproteins isolated from the serum of one patient with obstructive jaundice (patient M. F. of Table 1)*

The values for the flotation coefficients (s_r) were determined with a 1% (w/v) solution. The partial specific volumes ($\bar{v}$) were determined at 21.5°C. Values of $\bar{v}$ seem low compared with the results from various hydrodynamic measurements (Lee & Alaupovic, 1974; Nelson *et al.*, 1974) yielding the reciprocal buoyant densities of the particles. These magnitudes are, however, not necessarily equivalent to the ideal thermodynamic value of the partial specific volume, due to possible charge effects and/or preferential interaction with solvent or salt molecules, affecting the hydrodynamic properties of the particles. The values measured by the oscillator technique and given in this Table are equivalent to those obtained by weighing in the pycnometer, and are thus closely related to the thermodynamic value. Using the latter value of $\bar{v}$ in connexion with small-angle X-ray scattering (Laggner, *et al.*, 1976) yields a mol.wt. for lipoprotein-B of 2.4×10^6 .

Lipoproteins ...	Lipoprotein-X	Lipoprotein-Y	Lipoprotein-B _e	Lipoprotein-B (normal)
s_r , 1.063	14.6	18.3	7.5	6.1
$\bar{v}$ (ml/g)	0.961	0.964	0.952	0.947

1.063 g/cm³), they have slightly different partial specific volumes and different s_r (flotation coefficient) values. Table 3 lists the results determined with preparations from one individual. The pattern of these lipoproteins in the analytical ultracentrifuge is seen in Plate 4.

Discussion

The appearance of 'pathological' lipoproteins accompanied by marked hypercholesterolaemia and hypertriglyceridaemia in patients with obstructive liver disease has been noticed and investigated by numerous laboratories [for a review, see McIntyre *et al.* (1975)]. The metabolism of lipoproteins in general and the interconversion of lipoproteins of various density classes into each other in particular seems to be poorly understood at present under normal conditions and is a matter of speculation in diseased states. The chemical characterization of 'pathological' lipoproteins, an essential prerequisite

for uncovering metabolic pathways in health and in disease has been hampered by the difficulty of obtaining satisfactory amounts of samples and by the complexity of the lipoprotein and apolipoprotein system.

Seidel *et al.* (1969) succeeded in isolating and investigating 'pathological' lipoproteins occurring in obstructive jaundice: lipoprotein-X, a lipoprotein that is believed to be cholestasis-specific, attracted the attention of workers in many laboratories, but there are still some open questions about its exact protein and polypeptide composition. Another lipoprotein has been described, called ' β_2 -LP' according to its electrophoretic mobility (Müller *et al.*, 1974). Since the migration rate depends to a great extent on the agarose/agar ratio in the gel, we propose to call this lipoprotein lipoprotein-Y. This term should indicate both its pathological character and its relation to lipoprotein-X.

Lipoprotein ' β_2 -LP' (lipoprotein-Y) has been purified previously (Müller *et al.*, 1974) by a combined

procedure involving ethanol precipitation and immunoabsorption and desorption with NaSCN. Both ethanol and NaSCN can be considered as denaturing agents, especially for labile lipoproteins. We therefore developed another method to isolate 'pathological' lipoproteins. The separation procedure has the additional advantage of wider practicability, since immunoabsorbers loaded with anti-(lipoprotein-X) are not always available. Further, our method separates all the different 'pathological' lipoproteins in one single operation. Instead of using stepwise elution with 0.1–0.25M- and 0.65M-potassium phosphate buffers, a gradient elution with a linear gradient ranging from 0.1 to 0.65M-potassium phosphate was even more effective in giving further subfractionation (results not shown). This latter method, however, has the disadvantage of resulting in more-dilute fractions. In these subfractions lipoproteins differing in their antigenic components from peaks I-2, II-2 and III-2 have not been observed so far. The only differences were noticed in the molecular size and the lipid/protein ratio. The precipitation of LD-lipoproteins with phosphotungstate performed during the present investigation proved to be advantageous with larger samples. A simultaneous purification of 'pathological' lipoproteins carried out once by phosphotungstate precipitation, or alternatively by ultracentrifugation only, yielded lipoprotein-X, lipoprotein-Y and lipoprotein-B_c preparations indistinguishable in chemical composition, immunochemical behaviour and polypeptide pattern in polyacrylamide-gel electrophoresis from those precipitated with phosphotungstate. We therefore concluded that phosphotungstate does not alter the structure of those lipoproteins.

The data obtained with purified lipoprotein-X agree well with those published by other investigators. In addition we have proved that the antigens apolipoprotein-CI and apolipoprotein-AIII are situated at the surface of the lipoprotein-X particle, whereas other antigenic determinants of albumin, immunoglobulin G, and surprisingly of apolipoprotein-CII and apolipoprotein-CIII are hidden or masked. Lipoprotein-Y seems to be responsible to a major extent for the high triglyceride content of LD-lipoprotein in patients with obstructive jaundice; it does not migrate (or migrates only slowly under altered experimental conditions cathodically or anodically) in agar-gel electrophoresis and gives a precipitin line in the region of the application well. This lipoprotein can therefore only be verified by immunochemical methods, but not by precipitation methods, which are currently in use in many laboratories screening sera for lipoprotein-X.

Lipoprotein-Y consists of spherical particles of 40nm diameter, which in contrast with lipoprotein-X exhibit usually no formation of stacked discs in

negative-stain electron microscopy. Stacked discs have been observed only in few samples. By comparison with the published electron micrographs of LD-lipoproteins fractions isolated from phosphatidylcholine-cholesterol acyltransferase-deficient patients (Forte *et al.*, 1974; Glomset *et al.*, 1973), the abnormal lipoproteins formed in this metabolic disorder look much more like lipoprotein-Y than like lipoprotein-X. It therefore seems to be a very important question, which in our opinion deserves further clarification, whether lipoprotein-X really is formed in phosphatidylcholine-cholesterol acyltransferase deficiency, or whether the abnormal lipoprotein observed is lipoprotein-Y. The proposed isolation procedure should be applied to identify the abnormal LD-lipoprotein of phosphatidylcholine-cholesterol acyltransferase-deficient patients, since ethanol treatment may lead to aggregation or dissociation of some antigens of lipoprotein-B and lipoprotein-C. Gel filtration, on the other hand, in most of the cases gives some overlapping peaks and does not allow a clear-cut separation of pathological lipoproteins.

At the present stage of our knowledge it is an open question whether the abnormal lipoproteins found in the serum of patients with obstructive jaundice and/or phosphatidylcholine-cholesterol acyltransferase deficiency represent a catabolic product generated by the action of lipases on chylomicrons and VLD-lipoproteins, or whether they are formed as such in the liver or small intestine. Some observations in dietary studies on phosphatidylcholine-cholesterol acyltransferase-deficient patients (Glomset *et al.*, 1975) and measurements of lipoprotein lipase activity in patients suffering from liver disease (Müller *et al.*, 1974) point towards the possibility that the triglyceride-rich lipoproteins found in LD-lipoproteins (β_2 -LP or lipoprotein-Y) are derived from chylomicrons or VLD-lipoproteins. Further support for this hypothesis is the finding that these lipoproteins contain appreciable amounts of apolipoprotein-B linked to apolipoprotein-C by lipids. Nevertheless, lipoprotein-Y should be considered as a 'pathological' lipoprotein and not as a remnant of chylomicron or VLD-lipoproteins catabolism, since it differs considerably not only in its lipid composition but also in the polypeptide distribution from 'intermediate lipoprotein', which is augmented in Type III hyperlipoproteinemia (G. Kostner & J. Patsch, unpublished work).

It is also noteworthy that in cases where large amounts of lipoprotein-Y were found in the plasma, the lipoprotein-B_c fraction was unusually rich in triglycerides. This also abnormal lipoprotein shows in contrast with normal lipoprotein-B, appreciable amounts of apolipoprotein-C polypeptides which cannot be removed by column chromatography. Lipoprotein-X, however, a lipoprotein easy to differentiate from lipoprotein-Y and lipoprotein-B

in the electron microscope and by immunochemistry is very poor in triglycerides, and not even traces of lipoprotein-B or apolipoprotein-B can be found in the particle. Although experiments in which the common bile duct in mice was ligated failed to demonstrate the presence of lipoprotein-X-like lipoproteins in liver cells (Stein *et al.*, 1973), other experiments support the hypothesis that lipoprotein-X is of hepatic origin (Stakenberg *et al.*, 1974). Possibly the answer to this question could be obtained after clarification as to whether lipoprotein-X or lipoprotein-Y is the abnormal lipoprotein in phosphatidylcholine-cholesterol acyltransferase deficiency. Although activity of this enzyme can reach values below 50% of normal in patients with obstructive jaundice (Blomhoff *et al.*, 1974), it does not seem that the appearance of lipoprotein-X is necessarily due to altered phosphatidylcholine-cholesterol acyltransferase activity, since in some cases the apparent enzyme activity is normal, or even higher than in normal individuals. Lipoprotein-Y, on the other hand, is more likely to be formed in patients with phosphatidylcholine-cholesterol acyltransferase deficiency, since the lipid and/or polypeptide exchange between HD-lipoproteins and VLD-lipoproteins can be considered to be restricted. Further work aimed towards a more precise characterization of the lipoprotein constituents in all kinds of diseases and metabolic disorders will be necessary to answer these important questions.

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