ABNORMAL LOW DENSITY LIPOPROTEINS OCCURRING IN EXPERIMENTAL CHOLESTASIS IN THE RAT

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1. Introduction

The characteristic elevation of plasma free cholesterol and phospholipids in patients with biliary obstruction is partly due to the appearance of an abnormal lipoprotein called LP-X [1,2]. Cholestasis is also often accompanied by hypertriglyceridemia, and the major amounts of the triglycerides have been attributed to the triglyceride-rich abnormal LDL called variously β_2-LP [3] or LP-Y [4].

As in human cholestasis, ligation of the common bile duct in rats produces an elevation of plasma free cholesterol and phospholipids mostly due to the presence of LP-X [5]. Hypertriglyceridemia is also found in bile duct-ligated rats [5]. Here, the occurrence of an abnormal LDL rich in triglyceride in the cholestatic rat plasma is described.

2. Materials and methods

Male Wistar rats (300–350 g) were fed a standard laboratory diet ad libitum. The animals were fasted overnight prior to blood collection. Blood was drawn from the aorta under ether anesthesia in the presence of Na_2-EDTA (1 mg/ml). The blood of cholestatic rats was drawn 44–48 h after ligation of the common bile duct. Plasma was promptly isolated at 4°C and DTNB was added 0.4 mg/ml final conc.

The solvent density of pooled plasma was raised to 1.225 g/ml by adding solid KBr [6]. The solution was centrifuged at 156 500 × *g* for 40 h at 15°C using RP65T rotor in a Hitachi 65P ultracentrifuge. Whole lipoproteins were isolated by pipetting. The whole lipoprotein solution (4–5 ml) was applied to an agarose gel chromatography column (1.9 × 95 cm) which consisted of Bio-Gel A15m, 100–200 mesh (Bio-Rad Labs., Richmond, CA) and eluted as in [5]. The absorbance (or scattering) at 254 nm of the effluent was continuously monitored.

Free cholesterol and cholesteryl esters were determined by an enzymatic method as in [5]. Triglycerides were determined as in [7] and phospholipids by the method in [8]. Protein was determined as in [9]. Agarose gel electrophoresis was performed by a modification [5] of the method in [10].

Electron microscopy was performed on the lipoproteins after negative staining at room temperature with 2% phosphotungstic acid (pH 7.2) [5].

Abbreviations: LP-X, lipoprotein-X; LP-Y, lipoprotein-Y; β_2-LP, β_2-lipoprotein; HDL, high density lipoprotein(s); LDL, low density lipoprotein(s); VLDL, very low density lipoprotein(s); DTNB, 5,5′-dithiobis-2-nitrobenzoic acid

3. Results and discussion

In bile duct-ligated rats, signs of obstructive jaundice were observed as judged from the increase of plasma bilirubin and all the plasma lipids, mainly phospholipids and total cholesterol, ~50% of the latter being free cholesterol. Figure 1 shows the elution patterns of the plasma lipoproteins from bile duct-ligated rats and from control animals. Peak I contained VLDL, and LP-X was also eluted in this region along with VLDL in the cholestatic plasma. LDL (peak II) appeared as a small shoulder close to HDL (peak III) in the control rat plasma, reflecting the low LDL level in rat plasma. In the cholestatic rat plasma, there appeared a large peak II in contrast with the control, and hence this peak II lipoprotein was designated cholestatic LDL here.

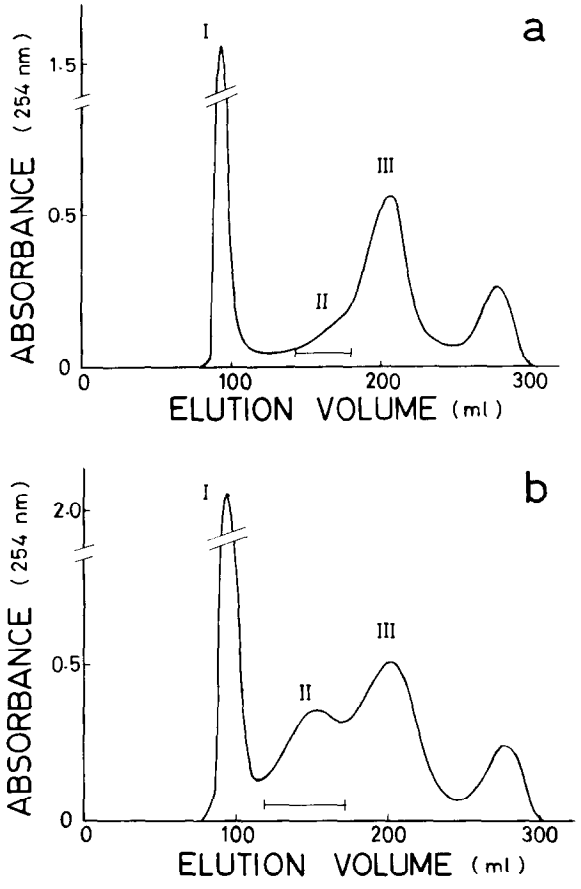

Fig.1. Elution patterns of plasma lipoproteins on a column of Bio-Gel A15m: (a) normal rat; (b) bile duct-ligated rat.

The fractions corresponding to peak II as indicated by the bars in the figure were pooled, concentrated and purified by further gel filtration through the Bio-Gel A5m column (1.5 X 95 cm), and the purified lipoproteins were subjected to further analyses. Table 1 shows the chemical composition of the

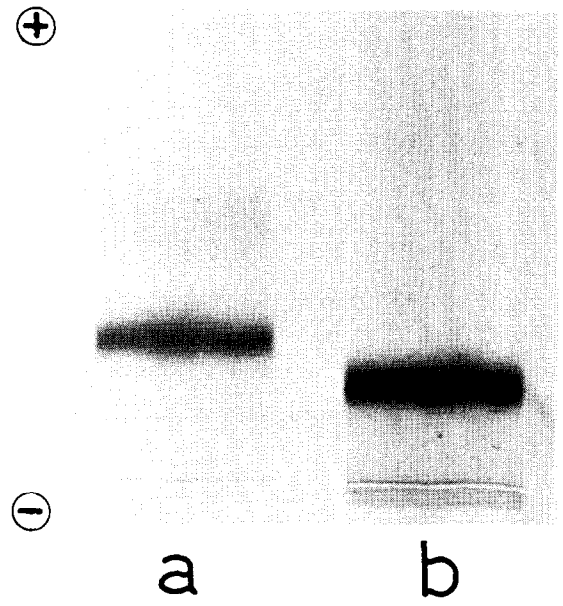

Fig.2. Agarose gel electrophoresis of: (a) normal LDL; (b) cholestatic LDL.

cholestatic LDL and the normal one. The cholestatic LDL is characterized by its high content of triglyceride and contains smaller amount of cholesteryl ester than the normal LDL.

In agarose gel electrophoresis, the cholestatic LDL migrated more slowly than the normal LDL (fig.2) and this electrophoretic property was different from that of VLDL in spite of the high content of triglyceride in the cholestatic LDL. Figure 3 shows the electron micrographs of negatively stained preparations of the cholestatic and the normal LDL. In the cholestatic LDL fraction, disc-like particles were seen infrequently but most of the particles appeared spherical in shape. The diameter of the cholestatic

Table 1
Chemical composition of the cholestatic LDL and the normal LDL (%, w/w)

	Triglyceride	Cholesteryl ester	Free cholesterol	Phospho-lipid	Protein
Cholestatic LDL	29.2 ± 1.3	12.8 ± 0.7	9.4 ± 1.2	30.0 ± 5.5	18.6 ± 6.2
Normal LDL	14.2 ± 5.0	20.1 ± 1.3	6.6 ± 2.1	20.4 ± 6.7	38.7 ± 6.7
P	<0.05	<0.025	n.s.	n.s.	n.s.

Values are means ± SD of 3 experiments; *P* values were obtained by Student's *t*-test; n.s., not significant

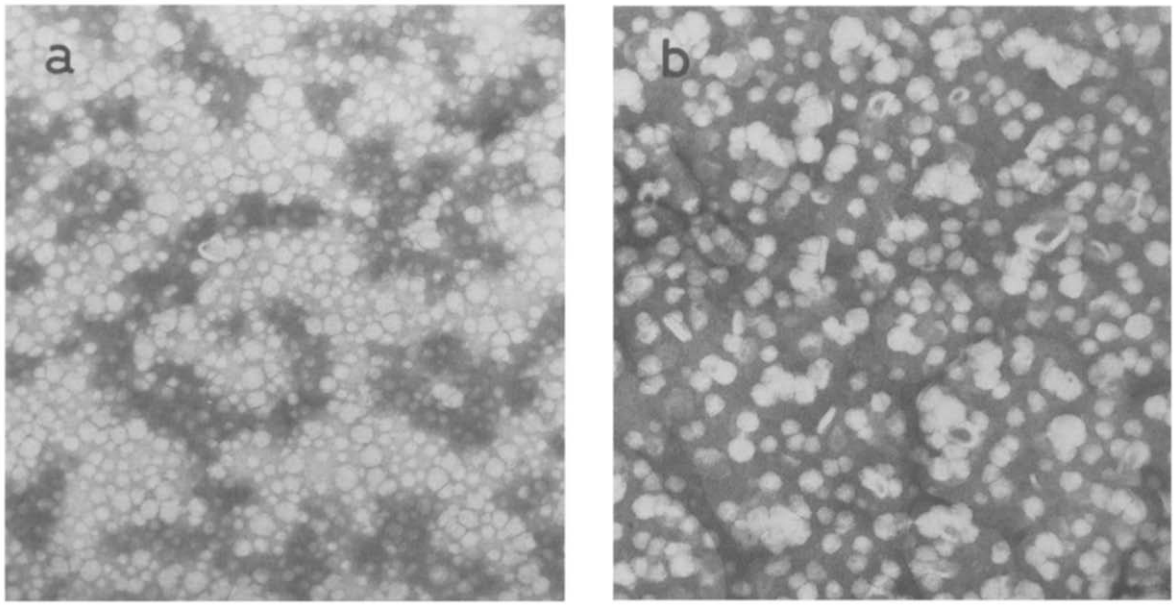

Fig.3. Electron micrographs of negatively stained: (a) normal LDL; (b) cholestatic LDL particles. (×115 500)

LDL particles was 26 ± 5 nm (mean ± SD, n = 132), while that of the normal LDL particles was 19 ± 5 nm (mean ±SD, n = 132).

These findings in electron microscopy were consistent with elution patterns of the lipoproteins (fig.1); the position where the cholestatic LDL appeared was slightly shifted to the left as compared with that of the normal LDL, although LDL was not eluted as a distinct peak in the normal rat plasma. Thus, the above cholestatic LDL is different from the normal LDL in electrophoretic property in agarose gel, in chemical composition, and in electron microscopic appearance.

This cholestatic LDL might be a kind of intermediate low density lipoprotein which has accumulated as a result of impaired liver function following cholestasis, since intermediate low density lipoproteins are suggested to be degraded efficiently in rat liver, which is responsible for the low LDL level in rat plasma [11]. Alternatively, this abnormal lipoprotein might be a counterpart of the abnormal LDL rich in triglycerides which was found in patients with obstructive jaundice and was called LP-Y [4].

Hypertriglyceridemia in the cholestatic rats would probably be due to the presence of a cholestatic LDL rich in triglycerides, although the degree to which the cholestatic LDL contributed towards the hypertriglyceridemia was not estimated in this experiment.

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