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ULTRACENTRIFUGAL CHARACTERIZATION OF A LIPOPROTEIN OCCURRING IN OBSTRUCTIVE JAUNDICE

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SUMMARY

The abnormal lipoprotein which characterises obstructive jaundice has been prepared in a purified form by two methods. On examination in the analytical ultracentrifuge, these preparations presented a single boundary which had an average flotation rate of 15.9 svedbergs in NaCl solution of density 1.063 g/ml.

It has been well documented that the characteristic elevation[2] of plasma lipoproteins in patients with obstructive jaundice is due to the presence of a low-density lipoprotein (LDL) of abnormal composition and properties[3-7]. Recently, Seidel *et al.*[7] have separated this abnormal compound from the accompanying normal LP-A and LP-B*** and have given it the designation LP-X. The latter, which has also been called "obstructive lipoprotein" by Switzer[5], differs immunochemically from LP-A and LP-B, and is distinguished by its high content of unesterified cholesterol and phospholipid, and by its low content of protein. The abnormal lipid composition of plasma from patients with obstructive jaundice[8] can therefore be ascribed to the presence of LP-X. Taken together, the LP-B and LP-X account for 98% of the protein content of the S_f 0-20 lipoproteins, which is the only part of the entire lipoprotein spectrum in which LP-X can be detected immunochemically. However, although the levels of lipoproteins in this class have been reported to be much increased in obstructive jaundice[2], the exact ultracentrifugal category to which LP-X belongs has not hitherto been identified.

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*** Abbreviations: LP-A, lipoproteins characterised by the presence of apo-lipoprotein A; LP-B, characterised by apo-lipoprotein B; LP-X, lipoproteins occurring in obstructive jaundice and characterised by the presence of apo-lipoprotein X. In this classification[1,7] each apo-lipoprotein is recognised by its specific immunochemical properties and by the identity of its terminal amino acids.

METHODS

Blood samples

Specimens of blood were drawn from two men suffering from obstructive jaundice secondary to carcinoma of the pancreas, in both of whom the obstruction was successfully relieved by surgery. From the first patient a specimen of about 100 ml, which will be designated sample 1, was taken just prior to the operation. From the second subject, three 10-ml samples were taken, one on the day before operation and the others 11 days and 17 days afterwards. These will be referred to as samples 2, 3 and 4, respectively. In each case the blood was drawn after an overnight fast, allowed to clot for 2 h in glass at 37°, and the serum separated by low-speed centrifugation.

Fractionation of lipoproteins

Pure samples of LP-X were prepared by two methods:

(1) Ten ml of serum sample 1 were subjected to the separation procedure described by Seidel *et al.*[7] which was modified by reversal of the order in which the "Cohn fractionation" and heparin precipitation stages were performed. The precipitated specimens of pure LP-B and LP-X will be called samples 1(b) and 1(x) respectively. The former was re-dissolved in 10 ml of 0.02 *M* tri-sodium citrate at 0°, and neutralised with sodium bicarbonate[9]. Fraction 1(x) was dissolved, at 0°, by the addition of 0.2 ml of 10% tri-sodium citrate and 0.1 ml of 20% NaCl solution, and then diluted to 10ml with pH 7.7, 0.001 *M* Tris buffer. This solution was then dialysed, firstly for 48 h against 9.0% NaCl and then for the same time against 5% $BaCl_2$. The precipitate which formed was centrifuged off and the supernate dialysed for 48 h against 0.15 *M* NaCl. Both fractions 1(b) and 1(x) were finally dialysed against NaCl solution of density 1.063 g/ml and the lipoproteins concentrated by ultracentrifugation[10]. All the dialyses in this procedure were performed at 4°.

(2) In serum sample 2 the LP-B was removed by precipitation with the specific antiserum. About 1 ml of the serum was added to an equivalent amount (determined by prior titration) of rabbit anti-serum to human β-lipoprotein from which the rabbit lipoproteins had been removed by ultracentrifugation at density 1.21 g/ml. The mixture was incubated for 3 h at 37°, kept overnight at 4°, and the precipitate removed by low-speed centrifugation. The LP-X was then separated from the serum proteins by ultracentrifugation at density 1.063 g/ml (ref. 10). This fraction will be called 2(x).

Ultracentrifugal analysis

The method used was that of DeLalla and Gofman[10], with the following modifications. Samples 1, 1(b) and 1(x) were centrifuged in the Spinco 30.2 rotor under the conditions of the original method, but the coordinates of the schlieren patterns obtained from the analytical centrifugation were transferred to punched paper tape by means of a P.C.D. digital data reader (P.C.D. Ltd. Farnborough, England) and the flotation rates calculated on a suitably programmed computer. Samples 2, 2(x) and 4 were centrifuged at a density of 1.063 g/ml for 22 h in the Spinco 40 rotor at 40000 rev/min. In these cases the schlieren patterns were measured with a microscope, the flotation rates being calculated[11] from the equation $d\ln x =$

sw^2dt. In all cases the flotation rates were corrected for concentration dependence, using the coefficient $k = 1.61 \times 10^{-4}$ (mg/100 ml)$^{-1}$ as quoted by DeLalla and Gofman[10].

Immunological analysis

Immunoelectrophoresis was performed in 1% agar (Special Agar-Noble, Difco Laboratories, Detroit, Mich.) according to the method of Grabar and Williams[12], employing barbital buffer, pH 8.6 and ionic strength 0.05. Rabbit anti sera to human α- and β-lipoprotein and to human serum album in obtained from Behringwerke AG (Marburg an der Lahn, Germany), which gave a single precipitin line with whole serum from normal subjects and showed no reaction with human serum albumin or LP-X. The rabbit anti-serum to purified LP-X was prepared as described previously[7].

RESULTS AND DISCUSSION

Both the pre-operative samples 1 and 2 had the high phospholipid conte... and the exaggerated ratio of unesterified to esterified cholesterol which are character istic of an acute obstructive jaundice[3-7]. These, together with other significant measurements are presented in Table I. In sample 3 the pathological features of

TABLE I

THE CHEMICAL CHARACTERISTICS OF THE SAMPLES OF JAUNDICE SERUM

Samples 1 and 2 were drawn from different patients the day before surgical relief of an obstruction of the bile duct. Sample 3 was taken from the same patients as sample 2, 11 days after the operation. All the measurements except the alkaline phosphatase are in units of mg/100 ml serum.

	Sample 1	*Sample 2*	*Sample 3*
Total cholesterol	605	394	230
Unesterified cholesterol	458	288	71
Phospholipids	723	726	351
Total bilirubin	10.8	17.2	7.5
Direct bilirubin	—	14.4	4.6
Alkaline phosphatase (K.A. units)	25.7	73.9	55.9

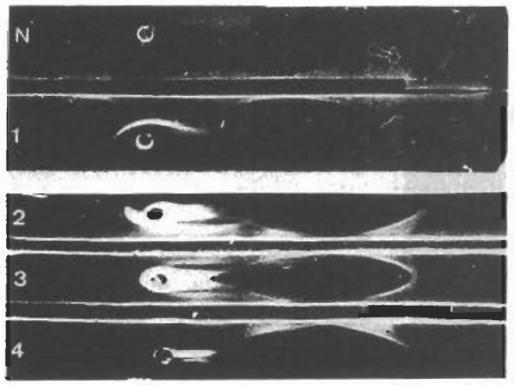

Fig. 1. Immunophoresis of samples of jaundice serum on agar gel. In each case The upper trough contained anti serum to purified LP-X: the two lower troughs contained mixed anti-sera to α-and β-lipoproteins, albumin and purified LP-X. Sample N was serum from a healthy person: samples 1, 2, 3 and 4 were put into the correspondingly marked holes. Migration was from left to right.

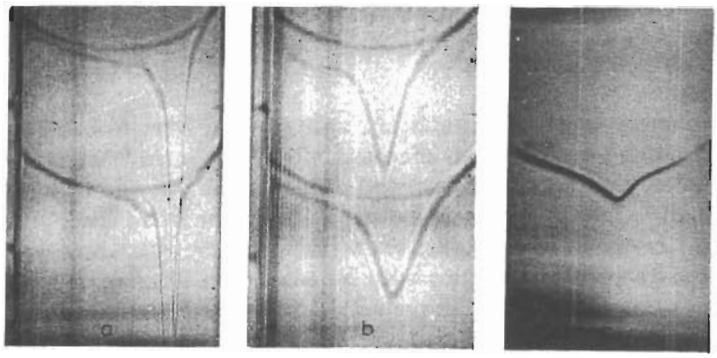

Fig. 2. Analytical centrifugation of the low-density lipoproteins from jaundice sera. In each case flotation was in NaCl solution of density 1.063 g/ml, at 52640 rev/min at 26°. The photographs were taken 30 min after reaching full speed. Sample 1 is shown in the upper diagram of Fig. 2a, the lower diagram being of lipoproteins from a healthy person. Sample 2 and 4 are shown in Figs. 2b and 2c respectively.

the second serum had begun to disperse, following surgical relief of the obstruction, while in sample 4 the serum chemistry had effectively returned to normal.

On immunophoresis, samples 1 and 2 gave a strong precipitin reaction with anti-serum to LP-X (Fig. 1). In sample 3 this reaction had considerably diminished, and in sample 4 LP-X was no longer detectable.

The distribution of low-density lipoproteins in serum samples 1, 2 and 4 is shown in Fig. 2 (a, b, c). In both the pre-operative sera two major components were present. In sample 1 these had flotation rates of 7.9 and 17.2 S_f units, while in sample 2 the values were 8.4 and 16.9 respectively. By contrast, the post-operative specimen 4, in which LP-X was absent, showed only a single peak of S_f 7.3. This evidence that the more rapidly sedimenting substance was LP-X was confirmed by the following observations on the isolated lipoproteins.

The immuno-diffusion analysis presented in Fig. 3a shows that the purified

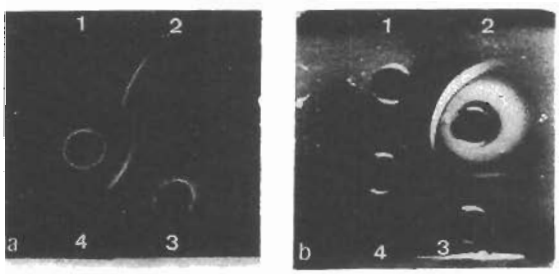

Fig. 3. Immunodiffusion analyses of lipoprotein fractions. The wells in Fig. 3a contained the following reagents: 1: anti-β-lipoprotein, 2: fraction 1(b), 3: anti-LP-X, 4: fraction 1(x). In Fig. 3b, the wells contained the following: 1: anti-β-lipoprotein, 2: serum sample 2, 3: anti-LP-X, 4: fraction 2(x).

lipoprotein fraction 1(b) gave a precipitin reaction with anti-serum to normal β-lipoproteins, but not with anti-LP-X. Fraction 1(x) however, reacted only with anti-LP-X. Ultracentrifugal analyses of these two fractions (Fig. 4a, b) showed that each contained a single component, the flotation rate of that in 1(b) being S_f 8.1, while

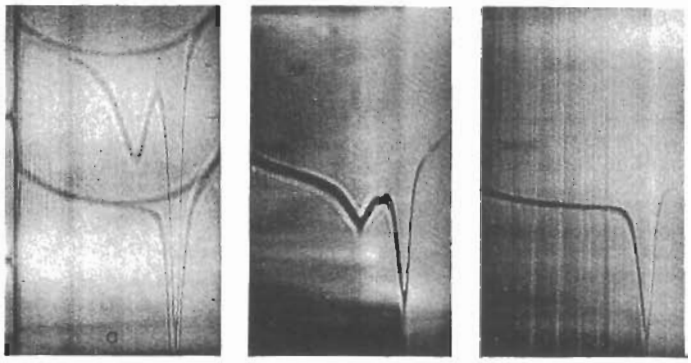

Fig. 4. Ultracentrifugal analysis of lipoprotein fractions from jaundice sera. Conditions of flotation were the same as for Fig. 2. The upper diagram of Fig. 4a shows the lipoprotein distribution in fraction 1(b), while that of Fig. 4b corresponds to fraction 1(x). Fraction 2(x) is shown in Fig. 4c.

that of 1(x) was S_f 15.9. From Fig. 3b it can be seen that sample 2 reacted strongly both with the anti-serum to β-lipoprotein and with anti-LP-X. However, the fraction 2(x), which was obtained by absorption with anti-β-lipoprotein, reacted only against anti-LP-X and, on ultracentrifugal analysis (Fig. 4c), yielded a single peak of S_f 15.8.

These centrifugal measurements show that the more slowly floating substance from samples 1 and 2 had a flotation rate which was a little greater than the average for the principal S_f 0–20 lipoprotein in healthy men (6.0–7.0 svedberg units[13,11]). In sample 4, in which the lipoprotein level had returned almost to normal, the flotation rate was closer to the average. However, this material not only had the immunological properties of a normal β-lipoprotein, but also behaved as one during the "Cohn fractionation" and the precipitation with heparin which form part of the isolation of LP-X[7]. By these criteria therefore, the slower peak in the flotation pattern of the low-density substances from jaundice serum corresponds to a normal β-lipoprotein (LDL).

In contrast to this compound, the LP-X had a slightly lower flotation rate when isolated than it had in the original mixture. The reason for this is obscure. This isolated lipoprotein gave no precipitin reaction with anti-serum to the normal β-lipoprotein and, although having an hydrated density less than 1.063 g/ml, was characteristically found with the high density lipoproteins in Cohn fraction IV+V+VI.

It may therefore be concluded that, although the plasma concentration of both the S_f 0–12 and S_f 12–20 lipoproteins may be greatly elevated in obstructive jaundice, it is the S_f 12–20 fraction that contains the immunochemically abnormal compound, and that this has a flotation rate near the range S_f 16–17.

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