UPTAKE OF LP-X AND ITS EFFECT ON THE ACTIVITY OF HMG-CoA REDUCTASE

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Cholestasis causes many lipid and lipoprotein abnormalities. Some of the abnormalities encountered in cholestatic liver disease have been recognized for over a century, while others have only recently been described. They can be summarized as follows:

1. Hypercholesterolemia.
2. Increased ratio of free to esterified cholesterol.
3. Hyperphospholipidemia.
4. Normal or elevated levels of triglycerides.
5. Increased concentration of LDL.
6. Normal or elevated levels of VLDL, often with β-mobility during electrophoresis.
7. Normal or decreased levels of HDL and also abnormal composition of HDL.
8. Often an accumulation of IDL.
9. Often a decrease in post-heparin lipolytic activity, primarily of hepatic triglyceride lipase.
10. Elevation or decrease of lecithin:cholesterol acyltransferase activity (LCAT-activity).
11. Appearance of lipoprotein-X (LP-X).

Of all these changes the presence of LP-X is the single characteristic feature of cholestasis.

The appearance of LP-X in plasma has also been noted after bile duct

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ligation of experimental laboratory animals, such as rats, dogs and pigs or after insertion of the common bile duct into the venous system (Seidel *et al.*, 1976). This abnormal lipoprotein floats at the density of ϱ 1.063 g/ml and is characterized by a high content of unesterified cholesterol and phospholipids. It contains only traces of esterified cholesterol and triglycerides. Apo-B, the main apoprotein of LDL, is absent. It exists in the form of a vesicle with a core of albumin together with the neutral lipids. The LP-X lipid precursors are synthesized in the liver and normally excreted into bile. During cholestasis this material refluxes into the plasma to form LP-X (Wieland and Seidel, 1980). It has not yet been established which organ removes LP-X from the circulation and catabolizes LP-X. It is also unclear, why in cholestasis the circulating LP-X, although it is rich in unesterified cholesterol, does not depress cholesterol biosynthesis in the liver which is known to be elevated in cholestatic liver disease.

METHODS AND RESULTS

Isolation and labelling of LP-X

In order to establish the site of LP-X uptake, LP-X was isolated from human bile by a combination of ultracentrifugation and Cohn fractionation (Manzato *et al.*, 1976). After checking its purity by chemical, electrophoretic and immunochemical analysis it was iodinated in the albumin moiety with ^{125}iodine by a standard iodination technique. It was then dialyzed and re-centrifuged for 20 hours at ϱ 1.065 g/ml to remove any traces of free iodine and albumin. Before use the preparation was again dialyzed and checked for purity.

Clearance of LP-X from the plasma

For *in vivo* experiments rate were anaesthetised with Evipan natrium and 1 ml ^{125}I LP-X (about 2–3 mg of free cholesterol) was injected into the saphenous vein. As seen in Fig. 1, LP-X disappears very rapidly from the circulation. The disappearance of ^{125}I LP-X is similar to the long-term decay curve obtained in rats which have been injected with unlabelled LP-X (Seidel *et al.*, 1976). Since ^{125}I LP-X is labelled in its albumin moiety it was necessary for control experiments to examine the kinetics of ^{125}I albumin removal. Albumin was labelled with ^{125}I by the same procedure as used for the preparation of ^{125}I LP-X and subjected to similar treatment. Rats were injected with ^{125}I albumin in concentrations of albumin corresponding to those found in the amount of ^{125}I LP-X injected. As seen in Fig. 2 removal of ^{125}I albumin does not follow the kinetics of removal of ^{125}I LP-X. Even the injection of higher concentrations of ^{125}I albumin did not alter its rate of removal from the blood.

Uptake of LP-X

Measurement of ^{125}I activity in various organs after the administration of

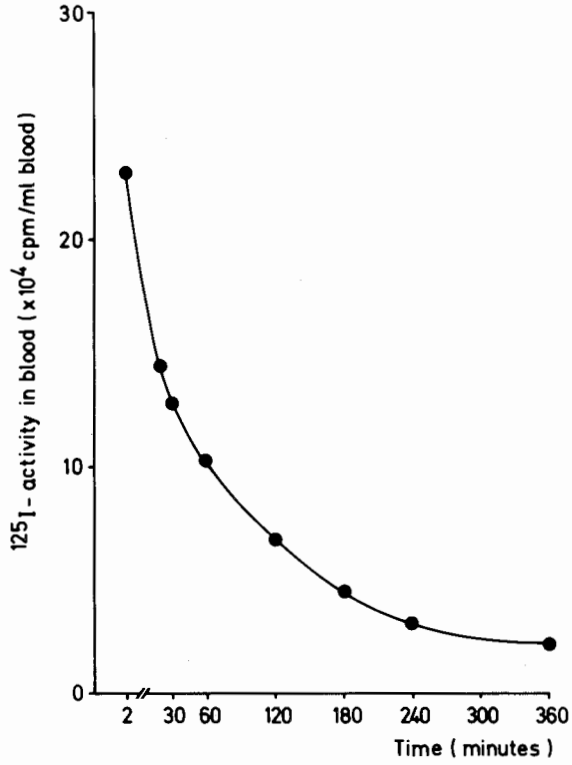

Fig. 1. Kinetics of removal of ^{125}I LP-X from blood. Rats were injected with 1 ml of ^{125}I LP-X in the saphenous vein and blood was removed at various time intervals to measure ^{125}I radioactivity.

^{125}I LP-X shows that most of the radioactivity is found in the spleen. Thus when rats were injected with ^{125}I LP-X the amount of radioactivity found in the spleen was seven-fold greater on a g wet weight basis than that found in liver at 60 min after injection. Measurement of the radioactivity at various time intervals shows that this high activity in spleen was maintained even 24 hours after injection of ^{125}I LP-X. A very small amount of radioactivity was found in other organs, such as pancreas, kidney, lung, heart and muscles. In contrast, after injection of ^{125}I albumin a small but equal amount of radioactivity was found in the liver and spleen (Fig. 3). These results clearly show that LP-X is mainly taken up by the spleen in rats and that the amount of LP-X removed from the circulation by the liver is only a small fraction of that removed by the spleen. Experiments with isolated perfused livers show that when ^{125}I LP-X is added to the perfusion medium it is removed by the liver only to a small extent but within a few minutes. However when hepatocytes were isolated from these perfused livers it was observed that hardly any of the ^{125}I activity found in the liver was present in parenchymal cells. Instead it was mainly in the non-parenchymal cells. These *in vitro* experiments substantiate

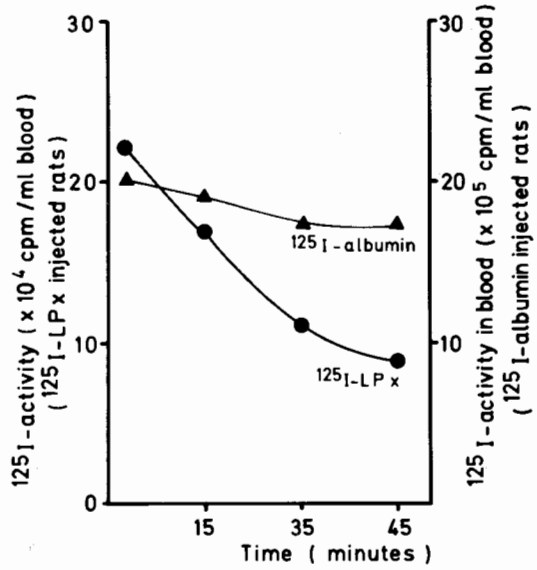

Fig. 2. Kinetics of removal of ^{125}I LP-X and ^{125}I albumin from blood. Rats were injected with 1 ml of either ^{125}I LP-X or ^{125}I albumin in the saphenous vein and blood was removed at various time intervals to measure ^{125}I radioactivity.

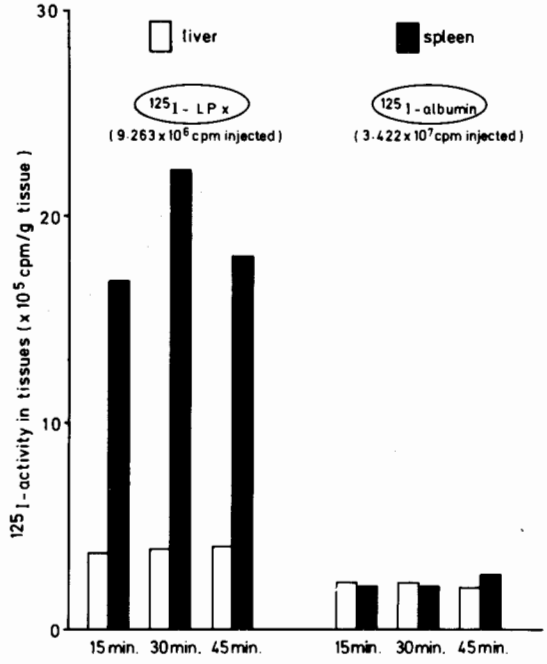

Fig. 3. Distribution of ^{125}I radioactivity in liver and spleen of rats after administration of ^{125}I LP-X or ^{125}I albumin (dpm/g wet weight tissue).

the results of the *in vivo* experiments, showing that the spleen and non-parenchymal liver cells remove LP-X from the circulation.

LP-X and hepatic HMG-CoA reductase activity

Cholestasis hypercholesterolemia is accompanied by increased activity of HMG-CoA reductase in the liver – an unusual metabolic situation In order to investigate the effect of LP-X as a predominant factor responsible for the hypercholesterolemia in cholestasis on the hepatic activity of HMG-CoA reductase, livers were isolated from rats and perfused with Krebs-Ringer HCO_3 medium containing 10% Hb and 2.5% albumin. After an equilibration period of 30 min LP-X was added to the medium to give a free cholesterol concentration in the medium corresponding to that found in rats after bile duct ligation. No inhibition of the activity of HMG-CoA reductase was observed in the liver microsomes by addition of LP-X during perfusion (Fig. 4). The continuous increase in the activity of HMG-CoA reductase in the liver noticed during perfusion in the presence of LP-X is more than that observed in its absence. When isolated livers are perfused with lipoprotein free medium they show a continuous increase in the activity of HMG-CoA reductase, probably due to loss of cholesterol from the liver cells (Cooper, 1976).

In order to establish whether the cholesterol available in the form of LP-X is capable of inhibiting the activity of HMG-CoA reductase of microsomes from liver we incubated isolated microsomes for 20 min with varying concentrations of LP-X. As Fig. 5 shows, LP-X in increasing concentration inhibits

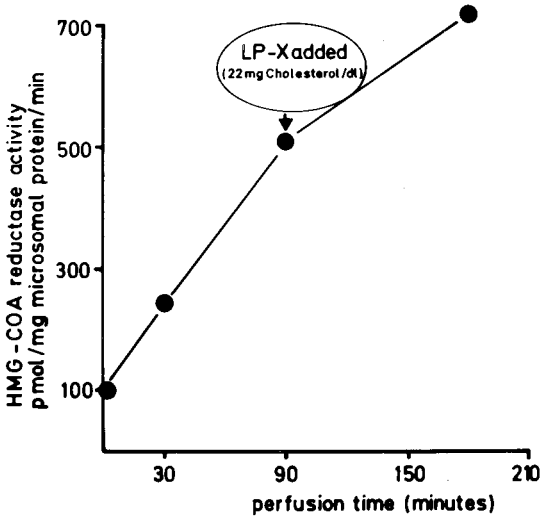

Fig. 4. Effect of LP-X on HMG-CoA reductase activity in isolated perfused livers. Livers were perfused with Krebs-Ringer HCO_3 medium containing 10% Hb and 2.5% bovine serum albumin. LP-X was added to the perfusion medium at 30 min. Liver samples were removed at various time intervals and microsomes were isolated for measurement of HMG-CoA reductase activity.

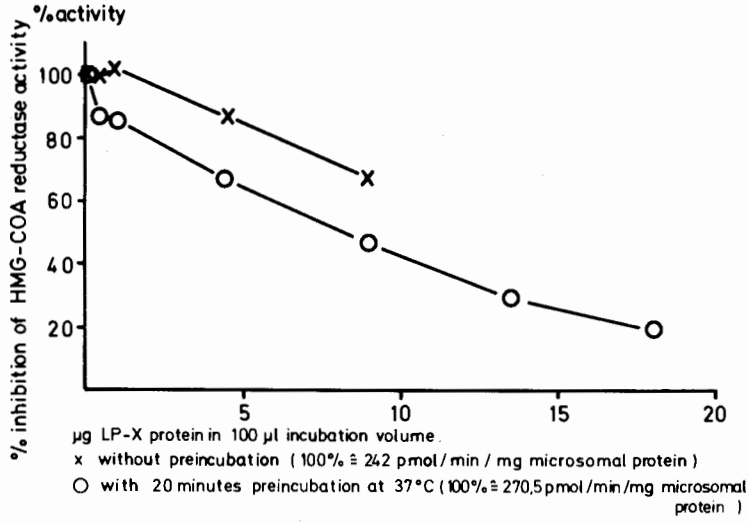

Fig. 5. Inhibition of HMG-CoA reductase activitiy in isolated liver microsomes by varying concentrations of LP-X.

the activity of HMG-CoA reductase in isolated microsomes to as much as 20% of control values. The failure of LP-X to do so in isolated perfused livers may be due to the inability of LP-X to enter the parenchymal liver cells, a phenomenon which may be caused by the structure and/or apoprotein composition of LP-X. In contrast the enzyme is inhibited in isolated microsomes since no permeability problems are encountered.

HMG-CoA reductase activity in lymphocytes of patients suffering from hepatic liver disease

Table 1 summarizes the activities of enzymes in serum and HMG-CoA

Table 1. Serum clinical chemistry and activity of HMG-CoA reductase in lymphocytes from cholestatic patients.

	Serum →							
Patient	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LP-X (U/l)	γ-GT (U/l)	AP (U/l)	GOT (U/l)	GPT (U/l)	Lymphocytes HMG-CoA reductase (pmol/mg protein/h)
W. L. ♂	215	259	+ +	372	369	78	120	o
B. M. ♀	75	54	+	106	258	35	74	11.5
B. E. ♂	469	139	+ + +	918	1500	44	72	1.3
Controls	150–260	50–170	–	≤18 ♀ 28 ♂	≤190	≤18	≤22	54.8

reductase in lymphocytes of cholestatic patients. In the patients with LP-X positive plasma the activity of HMG-CoA reductase is greatly diminished (0–11 pmol/mg protein/hour) in cholestatic patients as compared with 54 pmol/mg protein/hour in controls. These results suggest that LP-X or some other factors suppress the activity of HMG-CoA reductase in lymphocytes.

It is tempting to interpret these results in the light of the results obtained from the *in vivo* experiments performed with rats. LP-X is either taken up by the lymphocytes directly or after passage through the spleen and inhibits the activity of HMG-CoA reductase in the lymphocytes. Further experiments, however, are necessary to elucidate the mechanisms underlying the negligible or low activity of HMG-CoA reductase found in lymphocytes from cholestatic patients.

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