preparations isolated to date were activated by apolipoprotein A-I and to a limited extent by apolipoprotein C-I (Albers et al., 1979; Chung et al., 1979) and possibly by apolipoprotein D (Kostner, 1974). The trout and other species of fish have been shown to possess a plasma lipoprotein system which resembles that of mammals (Skinner & Rogie, 1978; Skinner & Youssef, 1980) though it would appear that a greater proportion of cholesterol and cholesteryl ester is found in fish. The aim of the work described in this communication was to further characterize the acyltransferase activity present in the plasma of rainbow trout and to determine the influence of exogenous factors such as nutritional status, diet and seasonal change on its activity.

Immature rainbow trout (Salmo gairdneri R.), 200-300 g, were obtained locally and maintained in well aerated freshwater aquaria until required. These fish were used as a source of plasma for the experiments in which the acyltransferase activity was partially purified and in those carried out to study the effects of starvation and dietary modification on this activity. In the seasonal study mature female trout, 1000-2000 g, were obtained from the Institute of Aquaculture, University of Stirling, Scotland, U.K. When required, fish were removed from the holding tank, anaesthetized with 0.03% (w/v) ethyl m-aminobenzoate (MS 222) and bled via the caudal vein by using a arterial catheter. The plasma lipoproteins were removed and all activities are given as d.p.m. of cholesteryl ester conversion of the lipoprotein layer at the top of the tube) was removed, except for 4°C. The plasma lipoproteins were removed and all activities are given as d.p.m. of cholesteryl ester formed as a percentage of the d.p.m. of total cholesterol.

Preliminary experiments showed that the rate of cholesterol esterification was linear for up to 5 h at 37°C (0-20% conversion) and that this enzyme was maximally active at 40°C. The effects of pH and of the total plasma cholesterol concentration on the activity of this enzyme were not investigated, since the influence of these parameters on the activity were dictated by the composition of the trout plasma.

The effects of the SH group inhibitor, DTNB, on the plasma activity were also studied. No inhibition of activity was observed over the range of final (assay) concentrations 0-7.5 mM.

Partial purification of the acyltransferase activity was achieved by preparative ultracentrifugation followed by DEAE-cellulose chromatography. Trout plasma was adjusted to 1.21 g/ml with solid NaBr and centrifuged at 100 000g for 40 h at 4°C. The plasma lipoproteins were removed and the fraction of maximum activity (that immediately below the lipoprotein layer at the top of the tube) was removed, extensively dialysed against 10 mM-Tris/HCl, pH 7.4, loaded onto a DEAE-cellulose column (75 mm x 9 mm) equilibrated with the above buffer and eluted with a linear NaCl gradient. A fraction containing acyltransferase activity was eluted at an NaCl concentration of 0.20 M. It was found that the material that eluted in this peak was active only when either heat-inactivated plasma (50°C for 30 min) or trout apolipoprotein A-I (10 μg) were included in the incubation medium.

The effect of physiological status on the activity of the acyltransferase in the whole plasma of the trout was also investigated. A comparison was made between trout starved for 8 weeks and a matched group maintained on a standard diet. The acyltransferase activity was significantly higher (P<0.001) in the fed group (3.74±0.65, n = 6; mean ± S.E.M.) than the starved group (1.79±0.36, n = 6). However, no significant difference was found in the activities in those fish maintained on a high-fat diet (20% cod liver oil) for 8 weeks and those maintained on a low-fat diet (5% cod liver oil); the respective activities were 5.53±3.16 and 0.07±1.25, n = 7. Further, the acyltransferase activity remained unaltered for most of the year, except for a significant increase (P<0.001) during the spawning period (March).

These studies suggest that the trout contains a cholesterol esterifying activity that broadly resembles the mammalian LCAT. The behaviour of the trout enzyme on ultracentrifugation and on DEAE-cellulose chromatography and its requirement for apolipoprotein A-I closely resemble mammalian LCAT. However, in common with other teleosts (e.g. Salmo alpinus; Dannevig & Norum, 1979) the trout enzyme is insensitive to the SH group inhibitor DTNB. The decrease in cholesterol esterification on starvation when there is a diminished transfer of cholesterol between very-low-density lipoprotein and high-density lipoprotein and the increase during spawning when cholesterol is needed for steroid and membrane synthesis suggest that this enzyme performs a key function in controlling the levels of cholesterol and cholesteryl ester in trout plasma.

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Hypolipoproteinaemic effects of ciprofibrate

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Abbreviations used: VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Ciprofibrate, 2-[p-(2-dichlorocyclopropyl)phenoxy]-2-methyl-propionic acid, is an effective hypolipidaemic agent in both rat and man, lowering both total serum cholesterol and triacylglycerol concentrations (Arnold et al., 1979; Olsson & Oro, 1982). In hyperlipidaemic subjects ciprofibrate lowers VLDL- and LDL-cholesterol but increases HDL-cholesterol (Olsson & Oro, 1982, Illingworth et al., 1982). The present studies on sucrose-fed hyperlipidaemic rats were carried out to investigate the effects of ciprofibrate on
serum lipoprotein concentrations and VLDL turnover in vivo. Rats were fed a 70% sucrose diet for 4 days, after which they received either the same diet or the diet containing 0.01% (w/v) ciprofibrate. Serum lipid concentrations were decreased in the ciprofibrate-treated rats in agreement with Arnold et al. (1976), cholesterol by 54% and triacylglycerol by 52%. The cholesterol contents of the VLDL and HDL fractions were reduced by 73% and 34%, respectively.

The protein content of the VLDL fraction was reduced from 0.23 ± 0.06 mg/ml (mean ± S.D.) in control rats to 0.11 ± 0.06 mg/ml in ciprofibrate-treated rats. The protein content of the HDL fraction was reduced from 0.67 ± 0.12 mg/ml to 0.25 ± 0.11 mg/ml. Thus, whereas the cholesterol/protein ratio was reduced in the VLDL fraction, the corresponding ratio in the HDL fraction was approximately doubled.

To determine whether the decreased protein content of the VLDL fraction after ciprofibrate was due to an inhibition of synthesis or an effect on catabolism, the clearance of 125I-labelled VLDL was followed in rats after intravenous injection. 125I-labelled VLDL was prepared by the method of Mcfarlane as modified by Fidge & Pouls (1975).

Fig. 1(a) shows the disappearance of total radioactivity from the VLDL fraction is shown in Fig. 1(b). In both groups there is an initial rapid catabolism, over the first 20 min, which is believed to be due, in part, to transfer of apoproteins to HDL (Eisenberg & Rachmilewitz, 1973). The data show that the rate of clearance of radioactivity from the VLDL fraction is essentially similar in the control and ciprofibrate-treated rats. The results cannot account for the marked fall in VLDL concentration observed in ciprofibrate-treated rats.

This work therefore suggests that the change in VLDL-protein concentration produced on ciprofibrate treatment is principally brought about by a decrease in the rate of secretion of VLDL particles from the liver.


Purification of lecithin–cholesterol acyltransferase from human plasma

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LCAT is synthesized by the liver and secreted into plasma, where it catalyses the transfer of long-chain fatty acyl residues from the 2-position of phosphatidylcholine to the 3β-hydroxy group of cholesterol (Glomset, 1968). Most of the cholesteryl ester in plasma is formed by the action of LCAT, and the enzyme helps to maintain the composition and structure of plasma lipoproteins (Owen & McIntyre, 1982). LCAT appears to act preferentially on the surface of HDL particles, a process implicated in reverse cholesterol transport to return peripheral cellular cholesterol to the liver for catabolism (Glomset, 1968; Davis et al., 1982).

The discovery of familial LCAT deficiency has provided a clearer understanding of the metabolic role of LCAT