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The characterization of lipoprotein lipase isolated from the post-heparin plasma of the rainbow trout, *Salmo gairdneri* Richardson

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1. Intravenous injection of heparin into the trout resulted in the appearance in the plasma of a lipase with the properties of lipoprotein lipase. 2. The enzyme was purified to apparent electrophoretic homogeneity by means of heparin–Sepharose affinity chromatography. The enzyme was eluted with 1.5 M-NaCl and had a specific activity approx. 450-fold that of the post-heparin plasma. 3. The activity of the purified enzyme was inhibited by 1.0 M-NaCl and protamine sulphate and was stimulated between 3- and 8.8-fold by the addition of trout plasma. 4. The activity was strongly stimulated by trout very low density lipoproteins and to a lesser extent by high density lipoproteins. 5. The isolated enzyme fraction gave a single band on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and had an apparent subunit M_r of 63000. 6. These results suggest that the uptake of lipid by the tissues in the trout can occur by a process similar to that in mammals.

It has been established that a number of species of fish, including the trout (Skinner & Rogie, 1978; Chapman *et al.*, 1978) contain plasma lipoproteins with general characteristics and apolipoprotein compositions that broadly resemble those of mammals, including man. Chylomicrons are normally present in fish plasma only in very low concentrations, but their levels are greatly increased after subjection to a high-lipid diet (Rogie & Skinner, 1981). Little is known, however, concerning the mechanisms involved in the uptake of lipid by the tissues in fish, though the above observations would indicate, by analogy, that a process similar to that in mammals implicating lipoprotein lipase (EC 3.1.1.34) is likely to occur.

It has been reported that when trout or carp were force-fed with radioactively-labelled palmitic acid or tripalmitoylglycerol respectively, (Robinson & Mead, 1973; Kayama & Iijima, 1976), the label appeared initially in unesterified fatty acid in the plasma and was converted into lipoprotein triacylglycerol only after a period of some 3 h. These observations suggest that an alternative or additional mechanism of lipid uptake occurs in fish. However, preliminary studies have shown that trout post-heparin plasma contains a lipase activity with the characteristics of lipoprotein lipase (Skinner *et al.*, 1980). In the present investigations, this enzyme was isolated from trout post-heparin plasma and its properties and characteristics were shown to be similar to those of mammalian lipoprotein lipase. The results of these studies therefore suggest that lipid uptake in the trout can occur by a process similar to that in mammals.

Abbreviations used: VLD, LD and HD lipoproteins; very low-density, low-density and high-density lipoproteins, respectively, isolated from trout serum by flotation at densities 1.006, 1.085 and 1.21 g/ml as defined in the text.

Materials and methods

Chemicals

Acrylamide, *NN'*-methylenebisacrylamide, *NNN'N'*-tetramethylethylenediamine and cyanogen bromide were purchased from Eastman Organic Chemicals. Bovine albumin, essentially fatty acid free, glycerol trioleate, heparin (sodium salt prepared from porcine intestinal mucosa) and protein markers were from Sigma. Freund's complete adjuvant and Noble agar were from Difco and silicic acid was obtained from Mallinckrodt. All other reagents and solvents were of analytical grade, purchased from BDH.

Fish

Commercially farmed trout (*Salmo gairdneri* Richardson) (1.0–1.6 kg) were obtained from local fish farms and maintained in aerated fresh water at

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10°C for the settling period during which they were offered standard fish pellets.

Preparation of post-heparin plasma

Trout were anaesthetized by immersion in fresh water containing 0.03% ethyl-*m*-aminobenzoate methanesulphonate and were injected intravenously through the caudal vein with heparin (100i.u./kg body weight, sodium salt, dissolved in 0.2ml of 159mM-NaCl). The fish were allowed to recover in fresh water and after approx. 35–40min (or at the times indicated in the case of the time-course experiments; see the Results section) from the administration of heparin, they were re-anaesthetized. Blood was removed from the caudal vein by means of a syringe, and the fish were killed whilst still under anaesthetic. Plasma was isolated by centrifugation at 1500*g* for 10min at 10°C and after recentrifuging under the same conditions was dialysed against three changes of 25vol. of 0.40M-NaCl in 5mM-veronal buffer, pH7.4, at 4°C for 2h with vigorous stirring.

Purification of lipoprotein lipase from post-heparin plasma by affinity chromatography

Heparin–Sepharose was prepared by covalently binding heparin to Sepharose-4B (Pharmacia) as described by Klor *et al.* (1976) after activation of the gel with cyanogen bromide by the method of March *et al.* (1974).

The dialysed plasma was centrifuged at 15000*g* for 30min at 4°C and the clear supernatant was diluted with an equal volume of dialysis buffer. A volume of 15–25ml of the diluted post-heparin plasma was applied to a column (1cm × 12.5cm) containing heparin–Sepharose that had been equilibrated with 5mM-veronal buffer, pH7.4, containing 0.4M-NaCl. After application of the sample the column was eluted with 120–150ml of veronal buffer containing 0.6M-NaCl to enable the A_{280} to fall to the base line. The column was finally eluted with veronal buffer containing 1.5M-NaCl. A flow rate of 20ml/h was maintained and the separation was performed at 4°C. The column effluent was continuously monitored on a LKB Unicord II at 280nm and the A_{280} of individual fractions (1.5ml) was also read with a Unicam SP.500 spectrophotometer. Appropriate fractions were dialysed against three to five changes of 25vol. of 50mM-NH_3/HCl buffer, pH8.5, containing approx. 1 unit of heparin per ml, at 4°C for 2h to remove the NaCl before lipase assays were carried out.

Determinations of lipase activity

Lipoprotein lipase activity was determined by a modification of the method of Hernell *et al.* (1975). The final incubation mixture contained (per ml): 2μmol of [^{14}C]trioleoylglycerol (0.13μCi/μmol); 0.075mmol of Tris/HCl buffer, pH8.5; 6mg of gum arabic; 15mg of bovine serum albumin; 0.11ml of trout serum and 0.09mmol of NaCl. The [^{14}C]trioleoylglycerol was purified by silica gel chromatography (Lis *et al.*, 1961) immediately prior to preparation of the emulsion in order to remove contaminating free fatty acid. For the determination of salt-resistant lipase activity, trout serum was omitted from the incubation mixture and the concentration of NaCl was increased to give 1.0mmol/ml of final incubation mixture. For the determination of the optimum pH for the enzyme activity, a series of different incubation media of different pH values were prepared. In experiments designed to study the effect of different lipoprotein fractions on lipoprotein lipase activity, the serum was replaced by the appropriate lipoprotein. In characterization experiments, specific inhibitors were added to the incubation medium as described in the Results section.

For enzyme assay, 100μl of the above assay mixture and 100μl of enzyme solution were incubated at 37°C for 60min with shaking. The reaction was terminated and the free fatty acids were extracted by the two-phase partition system described by Belfrage & Vaughan (1969). Aliquots (0.5ml) of the upper phase were transferred to vials containing 5.0ml of Instagel (Packard) and the radioactivity was measured in a Packard liquid-scintillation counter.

Electrophoretic and immunological methods

Polyacrylamide-gel electrophoresis of post-heparin plasma and the purified enzyme was carried out according to the method of Davis (1964) except that no spacer gel was used and samples were applied in volumes of 20μl containing sucrose. For molecular weight determinations, electrophoresis was carried out in 7.5% polyacrylamide in the presence of sodium dodecyl sulphate according to the method of Weber *et al.* (1972). Proteins used for calibration were bovine serum albumin (68000), ovalbumin (43000), chymotrypsinogen A (25000) and cytochrome *c* (11000).

Immunodiffusion analysis was carried out by the technique of Ouchterlony (1949). The preparation of antisera to trout lipoproteins has been described previously (Skinner & Rogie, 1978).

Protein analysis

Protein was determined by the method of Lowry *et al.* (1951) as modified by Miller (1959), with bovine serum albumin as standard.

Preparation of trout lipoproteins

VLD, LD and HD lipoproteins were prepared from the serum of untreated trout by flotation at densities 1.020, 1.096 and 1.21g/ml as previously

described (Skinner & Rogie, 1978). The VLD lipoproteins and HD lipoproteins were washed once by a second centrifugation at the densities used for their initial separation for 18h and 42h respectively. The LD lipoproteins were re-centrifuged at *d* 1.085g/ml for 18h to remove any traces of HD lipoproteins. The purity of the isolated lipoproteins was determined by electrophoresis on polyacrylamide gels.

Results

Release of lipoprotein lipase into plasma after injection of heparin

Lipoprotein lipase activity could not be detected in trout plasma before the injection of heparin. Lipase activity was present at a low level 8min after injection and increased for approx. 35min from the time of injection; it thereafter remained at an approximately constant value for a further 20min (Fig. 1). Therefore in all subsequent experiments, fish were bled approx. 35–40min after the time of heparin administration.

The assay of the post-heparin plasma samples in the presence of 0.6M-NaCl demonstrated that no significant salt-resistant lipase activity was present at any of the points on the time course shown in Fig. 1. Further evidence for the tentative identification of the lipase as a lipoprotein lipase with the characteristics of the mammalian enzyme is shown in Table 1.

Since the trout lives at temperatures considerably below the body temperature of mammals, the optimum temperature of the fish enzyme was determined on the unfractionated post-heparin plasma. As seen in Fig. 2, optimum activity was obtained at 37°C and this temperature was therefore used in all assays.

Isolation of lipoprotein lipase from trout post-heparin plasma by affinity chromatography

When trout post-heparin plasma was dialysed against column buffer, fractionated by stepwise elution from a heparin–Sepharose column and dialysed to remove salt, all over a 48–54h period, no lipase activity was detectable in any of the fractions. Later investigations revealed that between 66 and 90% of the activity of the purified enzyme was lost on storage at 4°C in 12h. Addition of phenylmethanesulphonyl fluoride or dithiothreitol had no stabilizing effect on the enzyme. A scheme was therefore devised that minimized the duration of the isolation procedure while producing the maximum degree of separation of lipoprotein lipase from other plasma constituents. This was achieved by applying post-heparin plasma that had been dialysed for 2h against 5mM-veronal buffer, pH 7.4, containing

Table 1. *Effect of specific inhibitors on the lipase activity of trout post-heparin plasma*

The activity was determined in the normal assay system (as described in the Materials and methods section) and in a system containing inhibitors at the concentrations indicated using post-heparin plasma collected 40min after injection of heparin.

Inhibitor	Lipase activity (nmol of fatty acid/h per ml)
No inhibitor	157.3
NaCl (0.6M)	2.1
Protamine sulphate (0.3mg/ml)	25.3
Sodium deoxycholate (50mg/ml)	104.5

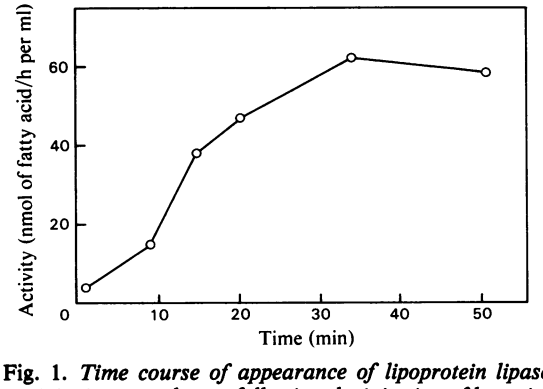

Fig. 1. *Time course of appearance of lipoprotein lipase activity in trout plasma following the injection of heparin*

Lipoprotein lipase activities were determined on plasma from blood samples withdrawn from a single fish at the times indicated. The assays were performed as described in the Materials and methods section.

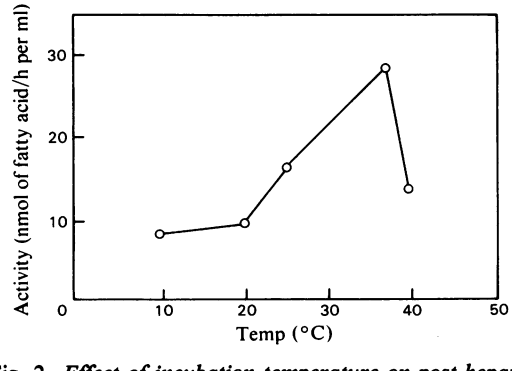

Fig. 2. *Effect of incubation temperature on post-heparin plasma lipoprotein lipase activity*

Trout post-heparin plasma lipoprotein lipase activity was determined at the temperatures indicated as described in the Materials and methods section.

Table 2. *Purification of lipoprotein lipase from trout post-heparin plasma by heparin–Sepharose chromatography*
The protein concentrations and enzyme activities refer to the protein-containing factors that were eluted from heparin–Sepharose in 5 mM-veronal buffer containing NaCl at the concentrations given.

Fraction	Volume of fraction (ml)	Total protein (mg)	Total activity (nmol of fatty acid/h)	Specific activity (nmol of fatty acid/h per mg)	Purification (-fold)	Recovery (%)
Post-heparin plasma	20	370	853	2.3	1.0	100
Eluate after loading	35	246	181	0.7	0.3	21
Eluate with 0.6 M-NaCl	135	147	367	2.5	1.09	43
Eluate with 1.5 M-NaCl	7.5	0.39	413	1045.0	454.0	48

0.40 M-NaCl, to the column and eluting over a 12 h period with buffers containing 0.60- and 1.5 M-NaCl, respectively, as described in the Materials and methods section, Pooled elution fractions were then dialysed against 50 mM-NH_3/HCl buffer, pH 8.5, containing 1 unit of heparin per ml, for 2 h and their lipoprotein lipase activities were determined. The results of a typical experiment are shown in Table 2. More than 99% of the protein applied to the column emerged during loading and by elution with 0.6 M-NaCl, while the 1.5 M-NaCl eluate had a specific activity that was 454 times that of the original post-heparin plasma. A significant proportion (38%) of the recovered activity, however, was eluted with buffer containing 0.6 M-NaCl. This loss of activity was reduced to approx. 3% in experiments in which the post-heparin plasma was applied to the column in buffer containing 0.15 M-NaCl with subsequent elution with 0.5 M-NaCl in place of 0.6 M-NaCl, but it resulted in contamination of the lipoprotein lipase in the 1.5 M-NaCl fraction subsequently collected with HD lipoproteins, as judged by immunodiffusion. VLD lipoproteins and LD lipoproteins were eluted with 0.15 M-NaCl and were therefore not present in the 0.5 M-NaCl eluate, but it was necessary to raise the NaCl concentration to 0.6 M to avoid contamination of the lipoprotein lipase with HD lipoproteins, even though this resulted in a loss of activity in the final fraction.

Polyacrylamide-gel electrophoresis of the 1.5 M-NaCl eluate showed the presence of a single band (Fig. 3).

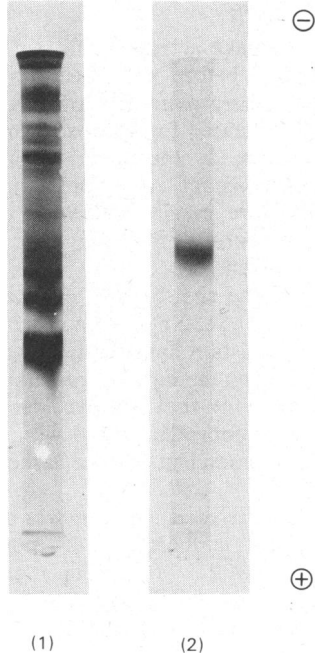

Fig. 3. *Electrophoresis on polyacrylamide gels of trout post-heparin plasma and of the 1.5 M-NaCl eluate from heparin–Sepharose chromatography*
Electrophoresis was carried out in 7.5% (w/v) acrylamide gels, without the use of spacer gels. Gels were stained for protein with Amido Black. (1) unfractionated trout post-heparin plasma; (2) eluate from heparin–Sepharose chromatography obtained with buffer containing 1.5 M-NaCl

Characteristics of the purified enzyme

The activity of the enzyme isolated in the 1.5 M-NaCl eluate was stimulated by an average of 6.1-fold (3.0 to 8.8 in five experiments) by the addition of trout serum to the incubation medium. In the presence of serum, the activity was strongly inhibited by protamine sulphate, 1 M-NaCl or sodium deoxycholate (Table 3). The optimum pH was between 8.0 and 8.5 (Fig. 4). These observations are consistent with the properties of lipoprotein lipase and distinguish the enzyme from other lipases.

The effect of adding individual trout lipoprotein fractions in place of serum on the activity of the purified post-heparin lipoprotein lipase is shown in Fig. 5. It is observed that VLD lipoproteins had the greatest stimulating effect, increasing the activity from approx. 16 to 132 nmol of fatty acid/h per ml at a protein concentration of 6 μg/assay, while HD lipoproteins caused a smaller but significant increase in activity (from 16 to 74 nmol of fatty acid/h per ml at a protein concentration of 20 μg/assay). Both of

Table 3. *Effect of specific activators and inhibitors on the activity of the purified trout enzyme*

The enzyme activity in the 1.5 M-NaCl eluate from heparin–Sepharose chromatography was determined in the standard assay medium and in the medium with the additions or deletion indicated.

Assay condition	Enzyme activity (nmol of fatty acid/h per ml)
Complete system	46.6
−Serum	10.8
+ Protamine sulphate (0.3 mg/μl)	7.1
+ 1 M-NaCl	2.1
+ Sodium deoxycholate (5%)	4.3

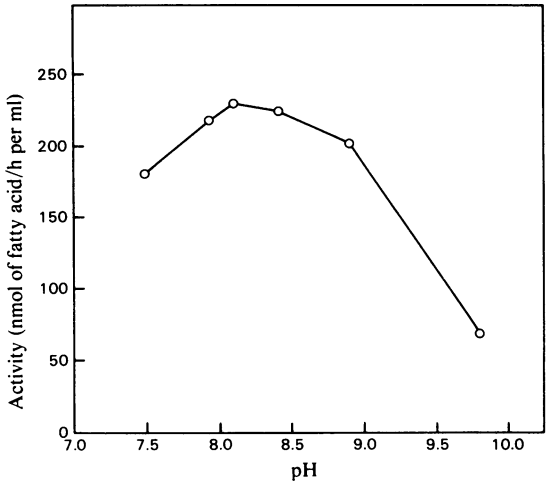

Fig. 4. *Effect of pH on the activity of purified trout lipoprotein lipase*

The enzyme isolated in the 1.5 M-NaCl eluate was assayed at the pH values indicated as described in the Materials and methods section.

these lipoprotein preparations appeared to be pure as judged by polyacrylamide-gel electrophoresis. The addition of preparations of trout LD lipoproteins, on the other hand, produced no significant increase in activity at protein concentrations up to 20 μg/assay, but at higher concentrations an increase in activity was obtained (60 and 80 nmol of fatty acid/h per ml at protein concentrations of 30 and 50 μg/assay, respectively). The preparation of LD lipoproteins used in these experiments was shown by polyacrylamide-gel electrophoresis to be very slightly contaminated with VLD lipoproteins; it was estimated that the stimulatory effects observed would have been obtained by the presence of contaminating VLD lipoprotein if the latter was present to the extent of 1%. The difficulties encountered in isolating

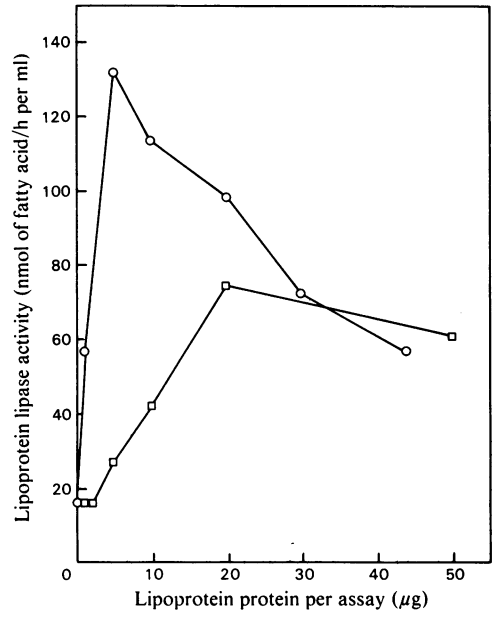

Fig. 5. *Effect of trout VLD and HD lipoproteins on the activity of purified lipoprotein lipase*

Increasing amounts of VLD (○) and HD (□) lipoprotein were added to the incubation mixture and the lipoprotein lipase activity was measured as described in the Materials and methods section.

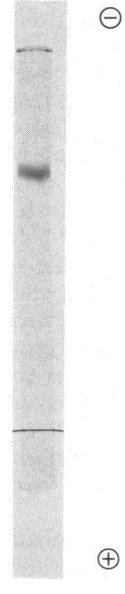

Fig. 6. *Electrophoresis on sodium dodecyl sulphate/polyacrylamide gels of purified trout lipoprotein lipase*

Electrophoresis of the 1.5 M-NaCl eluate from heparin–Sepharose chromatography was carried out in 7.5% (w/v) acrylamide gels in the presence of 0.1% sodium dodecyl sulphate, according to the method of Weber *et al.* (1972).

completely pure trout LD lipoprotein have been discussed previously (Skinner & Rogie, 1978). It is therefore concluded that VLD and HD lipoproteins stimulate the activity of lipoprotein lipase whereas LD lipoproteins probably have little or no effect.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

A single band was obtained when purified lipoprotein lipase was submitted to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (Fig. 6). A comparison with marker proteins indicated an apparent subunit M_r of approx. 63000. The same result was obtained when the electrophoresis was performed at three different protein concentrations, and also when reduction with mercaptoethanol was carried out for 0, 2 and 5min, suggesting that complete reduction without polypeptide degradation occurred under standard conditions.

Discussion

In the present studies, it has been demonstrated that intravenous injection of heparin into trout causes the release into the plasma of a lipase that shows many of the properties that are characteristic of mammalian lipoprotein lipase.

Thus the enzyme bound strongly to heparin, and when trout post-heparin plasma was applied to a heparin–Sepharose affinity column, the bulk of the lipase activity was eluted in the 1.5M-NaCl fraction, with little lipase activity eluting at lower concentrations of NaCl. This corresponds to the elution properties of lipoprotein lipase of human and rat post-heparin plasma (Augustin *et al.*, 1978) and of human milk (Hernell & Olivecrona, 1974). The activity of the lipase in the 1.5M-NaCl eluate was strongly inhibited by 0.6M-NaCl, protamine sulphate and sodium deoxycholate and the activity of the enzyme was considerably reduced when serum was deleted from the assay system, while addition of serum caused a 3–8-fold increase in activity. These features further identify the enzyme as lipoprotein lipase and distinguish it from salt-resistant (hepatic) lipase (La Rosa *et al.*, 1972). The trout enzyme showed a pH optimum of approx. 8.2, which is close to that of the mammalian enzyme (Hernell *et al.*, 1975).

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the active fraction eluted with 1.5M-NaCl revealed the presence of a single band which corresponded to an apparent M_r of approx. 63000. This is similar to the values of 62000–66000 obtained for lipoprotein lipase from bovine milk (Egelrud & Olivecrona, 1972), 60000 from chicken adipose tissue (Kompiang *et al.*, 1976), 67000 from human post-heparin plasma (Augustin *et al.*, 1978), 62000 from pig post-heparin plasma (Kuusi *et al.*, 1979) and 60000 from pig adipose tissue (Bensadoun *et al.*, 1974), though Chung & Scanu (1977) reported a minimum M_r of 34000 for the lipoprotein lipase isolated from rat heart.

From the above considerations it would appear that trout post-heparin lipoprotein lipase closely resembles the corresponding mammalian enzyme with respect to its general properties, including heparin binding and effect of activators and inhibitors and apparent subunit M_r. However, certain features were observed with the trout enzyme that differ from those of mammalian lipoprotein lipase.

A characteristic feature of lipoprotein lipase release by heparin administration in mammals, including man, is that high concentrations of the enzyme are produced almost at the moment the heparin enters the circulation (Nikkila, 1953). In the case of the trout, however, the activity of the enzyme in the blood increased gradually for approx. 35min and then remained at about the same level for the remaining 20min of the experiment. The reason for this slow response in the fish remains unexplained, though it may suggest a different mode of binding of the enzyme to the tissue capillary walls or may possibly be due to a slow turnover of blood in some parts of the vascular system (see Randall, 1970). A further regular feature was the low activity of the post-heparin plasma and also of acetone powders or extracts of fresh tissues; extracts of trout adipose tissue had similar activities to post-heparin plasma and these were less than 10% of the activity obtained from lactating guinea pig mammary gland (Robinson, 1963) prepared under the same conditions (A. M. Youssef & E. R. Skinner, unpublished observations). In addition, the trout post-heparin enzyme both in its crude form and especially after purification was considerably less stable than the mammalian enzyme. Although the rate of inactivation was somewhat reduced by the presence of heparin, the fact that addition of dithiothreitol, phenylmethanesulphonyl fluoride or glycerol had no significant affect in stabilizing the enzyme would suggest that the rapid loss of activity results from the instability of the enzyme molecule after its detachment from the capillary walls rather than to reduction of thiol groups or to proteolytic cleavage.

A further interesting difference between trout and mammalian lipoprotein lipase is the effect of different plasma lipoprotein fractions on the activity of the purified enzyme. The trout enzyme was strongly activated by trout VLD lipoproteins, while trout HD lipoproteins stimulated the enzyme to a small, though significant extent. This situation contrasts with that of the human post-heparin enzyme which is activated to a greater extent by HD lipoproteins than by VLD lipoproteins (Fielding,

1970), the activation being due to apolipoprotein C-II (LaRosa *et al.*, 1970) which is present as a constituent protein in both VLD and HD lipoproteins (see Eisenberg & Levy, 1975). Although a corresponding apolipoprotein has not as yet been positively identified in trout lipoproteins, an apoprotein with similar properties has been shown to be present in trout VLD and HD lipoprotein (Skinner & Rogie, 1978; Chapman *et al.*, 1978). Presumably the difference in the activating effects of VLD and HD lipoproteins in the trout and man is caused by a difference in the distribution of this apolipoprotein between the two lipoprotein fractions in the two species or by a difference in their availability due to differences in structure and integrity of the lipoprotein particles.

While the results of the present investigations demonstrate the presence of lipoprotein lipase in trout post-heparin plasma, salt-resistant lipase, which is also released into the circulation by heparin in the human (LaRosa *et al.*, 1972), appeared to be absent from trout post-heparin plasma. This is shown by the high degree of inhibition by 1 M-NaCl of lipase activity in unfractionated trout post-heparin plasma or in any of the heparin–Sepharose fractions and also by the lack of a lipase eluting with 0.7 M-NaCl in experiments where the column was fully equilibrated (A. M. Youssef & E. R. Skinner, unpublished work). The lack of salt-resistant lipase in the post-heparin plasma seems surprising, as the enzyme has been identified in trout adipose tissue (Skinner *et al.*, 1980). It is possible that the salt-resistant lipase is less readily released in the trout as a result of the factors discussed above.

The material that was eluted from the heparin–Sepharose column with 1.5 M-NaCl and which showed characteristics that are analogous to mammalian lipoprotein lipase appeared as a single band on polyacrylamide-gel electrophoresis, both in the presence and absence of sodium dodecyl sulphate. Although immunodiffusion experiments using antisera to trout VLD, LD and HD lipoproteins failed to detect the presence of these lipoproteins in the isolated material, the fact that the enzyme fraction showed some residual activity in the absence of added serum or activating lipoprotein suggests that it may contain a small amount of tightly-bound activating apolipoproteins. Furthermore, studies on mammalian post-heparin plasma have shown that the use of heparin–Sepharose affinity chromatography alone yields an enzyme fraction of which a major constituent is antithrombin (Östlund-Lindqvist & Boberg, 1977). The use of additional purification steps has yielded lipoprotein lipase preparations with specific activities of approx. 20000 and 37000 μmol/h per mg for the human post-heparin plasma and bovine milk enzymes respectively (Becht *et al.*, 1980; Iverius & Östlund-Lindqvist, 1976). The low specific activity of the trout enzyme preparations reported in this paper (see Table 2) may be due in part to its rapid rate of inactivation but could indicate that it may contain antithrombins as a major component which migrates in the same position as lipoprotein lipase on electrophoresis.

The finding that the intravenous injection of heparin into the trout releases an enzyme with the characteristics of lipoprotein lipase enables a better understanding to be gained of the processes that occur in the transport of lipid and the uptake of the latter by the tissue in fish. That fish possess a lipid transport system that is similar to that of mammals has already been indicated by the observations that trout plasma contains lipoproteins with characteristics, including apolipoprotein compositions, that are similar to their mammalian counterparts (Skinner & Rogie, 1978; Chapman *et al.*, 1978) and also that trout plasma contains chylomicrons which increase in concentration when the fish are maintained on a high-lipid diet (Rogie & Skinner, 1981). However, it has been observed that when trout or carp were fed with radioactively labelled palmitic acid or tripalmitoylglycerol, respectively, (Robinson & Mead, 1973; Kayama & Iijima, 1976), labelled fatty acid appeared initially in the plasma as unesterified fatty acid and was found as lipoprotein triacylglycerol only after an interval of some 3 h. A possible explanation is that lipid leaves the intestine as unesterified fatty acid (attached to a carrier protein) which is taken up by the liver, where lipoprotein synthesis occurs, and perhaps also by other tissues (see Cowey & Sargent, 1977). While further investigations are needed to determine the role and contribution of the additional fatty acid transport system that occurs in fish, the present studies demonstrate that the tissues of the trout are likely to have the ability to take up lipid circulating in the form of lipoprotein triacylglycerol by means of lipoprotein lipase.

A basic knowledge of the mechanisms of lipid transport and uptake in fish is of value in assessing the fate of associated lipid-soluble compounds such as hydrocarbons and carotenoid pigments which are of importance from the point of view of environmental pollution and the artificial rearing of fish.

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