Distribution and Characterization of the Serum Lipoproteins and Their Apoproteins in the Rainbow Trout (*Salmo gairdnerii*)†

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ABSTRACT: Two major lipoprotein classes, of β and α mobility, were detected in sera from adult rainbow trout upon immunoelectrophoresis. After taking into account the background density of trout serum (1.015 g/mL), these lipoproteins were fractionated by sequential preparative ultracentrifugation into the conventional density classes applied to the human substances, i.e., very low (d < 1.006 g/mL), low (d 1.006–1.063 g/mL), and high density (d 1.063–1.21 g/mL) lipoproteins (VLDL, LDL, and HDL, respectively). Immunological studies showed these fractions to be free of contaminating serum proteins. HDL was the major lipoprotein class (ca. 1500 mg/100 mL of serum), representing some 70% of the total substances of d < 1.21 g/mL. All trout lipoproteins displayed a high net surface charge on electrophoresis. The chemical composition of trout and human VLDL was alike, although the trout fraction was richer in polar components, an observation consistent with its smaller average particle size (<500 Å). Trout LDL were richer in protein and phospholipid and also smaller in size than the human fraction, suggesting that they mainly consisted of molecules with a hydrated density close to 1.063 g/mL. This suggestion was confirmed (1) by determination of the analytical ultracentrifugal distribution of the low-density substances and (2) by analysis of their distribution (on the basis of their β reactivity) following fractionation by density gradient ultracentrifugation, which showed that β-lipoproteins extended to a density of 1.078 g/mL. Dodecyl sulfate–polyacrylamide gel electrophoresis of the total apoproteins of VLDL and LDL indicated that their major component was of high molecular weight (>250 000.) This component was isolated from apo-VLDL and apo-LDL-2 (d 1.025–1.078 g/mL) by gel-filtration chromatography; its physical behavior upon electrophoresis and its amino acid composition indicate it to be a counterpart of human apolipoprotein B. Preliminary studies of HDL indicated that its two major apolipoproteins may resemble human apolipoproteins AI and AII. Major differences were noted in the complements of the low molecular weight, tetramethylurea-soluble protein components of trout and human lipoproteins. Finally, immunological cross-reactivity occurred between human VLDL and LDL and antisera to trout VLDL and to trout LDL. These data indicate that, despite the large evolutionary distance between trout and man, major elements of the structure of their serum lipoproteins and apolipoproteins are shared.

At the present time, there is a paucity of information on the mechanisms of lipid transport in nonmammalian vertebrates and particularly in fish species. Of late, however, Mills and co-workers (Mills et al., 1977) have reported detailed studies of the three major classes of serum lipoproteins in an elasmobranch, the shark *Centrophorus squamosus,* while Nelson and Shore (1974) have characterized the high-density lipoproteins and their major apolipoproteins in a teleost, the late prespawning pink salmon (*Oncorhynchus gorbuscha*). Some data are also available on the serum lipoproteins of the pacific sardine (*Sardinops caerulea girard*) (Lee and Puppione, 1972), spiny dogfish (*Squalus acanthias*) (Lauter et al., 1968), cod (*Gadus mohua* L.) (Skinner, 1973), dogfish (*Scyllium canicula*), and plaice (*Pleuronectes platessa*) (Mills and Taylaur, 1971).

Since several aspects of lipid digestion, absorption, transport, and storage in fish differ from those typical of mammalian species (Cowey and Sargent, 1977; Robinson and Mead, 1973; Tashima and Cahill, 1965), they appear to represent interesting models from which insight into the structure and function of serum lipoproteins in higher vertebrates, and particularly mammals, may be gained. The rainbow trout (*Salmo gairdnerii*), a teleost, has considerable appeal as an experimental animal for such studies, since it is readily available, may be bred with ease, is amenable to nutritional studies, and has a characteristic life cycle. In addition, trout are of interest to investigators of experimental atherosclerosis, since both nonanadromous rainbow and anadromous steelhead (also *S. gairdnerii*) commonly exhibit atherosclerotic lesions during sexual maturation and subsequent spawning (Moore et al., 1976; Mckenzie et al., 1978); these lesions primarily involve an intimal proliferation and in the case of the steelhead trout may completely regress upon return of the adult animal to the sea (Van Citters and Watson, 1968). Little information is presently available to account for this phenomenon, which apparently occurs in the presence of extremely high concentrations of HDL[1] (see Chapman et al., 1977a), and thus may conflict with recent suggestions that elevated HDL levels afford protection against atherogenesis, at least in man (Miller and Miller, 1975; Carew et al., 1976): studies of the trout lipoprotein system would appear fundamental to the eventual elucidation of its mechanism.

In the present report, we describe the characterization and

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[1] Abbreviations used: VLDL, very low density lipoproteins of density <1.006 g/mL; LDL, low density lipoproteins of density as defined; HDL, high density lipoproteins of density 1.063–1.21 g/mL; Apo-B, apolipoprotein B; EDTA, ethylenediaminetetraacetic acid. Apolipoprotein nomenclature is according to Alaupovic (1972).

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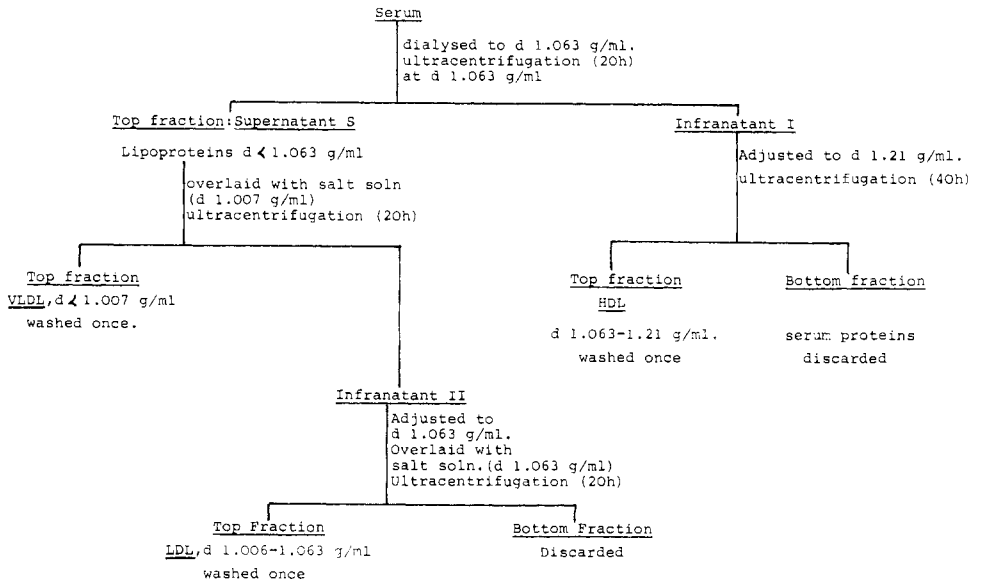

FIGURE 1: Scheme for the ultracentrifugal isolation of trout serum lipoproteins. For further details, see Materials and Methods.

some comparative aspects of the very low and low density lipoproteins from the sera of adult trout, together with the principal chemical and physical properties of their major apolipoprotein, which is a counterpart to apolipoprotein B in man. In addition, preliminary data on the HDL and their major apolipoproteins are presented.

Materials and Methods

Blood Specimens. Adult male and female rainbow trout (700–1000 g) were usually bled 8 h after feeding from the dorsal caudal vein. These fish had been commercially farmed and fed on a DS[2] diet; they were subsequently maintained in freshwater at 17 °C.

After clotting, serum was separated by low-speed centrifugation, and antibacterial agents were added (0.001% sodium merthiolate and 0.01% sodium azide). Each serum sample (up to 15 mL) was treated separately; samples were pooled only for the isolation of apolipoproteins. Since the trout egg lipoprotein is of $d > 1.21$ g/mL (Skinner and Rogie, 1977) (i.e., more dense than HDL), the sera from vitellogenic female fish were treated in the same way as that from males.

Serum Lipid Concentrations. The total triglyceride concentration of trout serum was determined by the colorimetric assay of Biggs et al. (1975), while that of cholesterol was estimated with the enzymatic kit of Boehringer Mannheim GmbH, the method being based on that of Roeschlau et al. (1974).

Isolation of Lipoproteins. (A) Sequential Ultracentrifugation. In the initial series of experiments, trout serum lipoproteins were isolated by sequential preparative ultracentrifugation (Havel et al., 1955) in the density intervals classically applied to the human substances (i.e. VLDL, $d < 1.006$ g/mL; LDL, d 1.006–1.063 g/mL, and HDL, d 1.063–1.21 g/mL). However, since determination of the nonprotein solvent density of trout serum by the equilibrium-dialysis technique (Jensen and Smith, 1976) showed it to be 1.015 g/mL, this procedure was employed in a modified form and is summarized in Figure 1. Thus, the density of the serum was raised to 1.063 g/mL by exhaustive dialysis at 4 °C against a NaCl solution of d 1.063 g/mL.[3] Aliquots (3 mL) of this solution were overlaid with 3 mL of d 1.063 salt solution in tubes of the Type 40.3 rotor and centrifuged at 40 000 rpm (114 500g) for 18–20 h in a Beckman L3-40 ultracentrifuge at 6 °C. After removal of lipoproteins of $d < 1.063$ g/mL in a volume of 1–2 mL by aspiration (supernatant S), the next 1 mL was discarded, and the density of the resuspended infranatant (I) was raised to 1.21 g/mL by the addition of an appropriate volume of a d 1.340 g/mL salt solution. Infranatant I (7 mL) was then overlaid with a salt solution (5 mL) of the same density (i.e., 1.21 g/mL) and centrifuged in a Type 40 rotor at 40 000 rpm (106 000g) for 48 h. The high-density lipoproteins (d 1.063–1.21 g/mL) could be removed in the top 2 mL after centrifugation under similar conditions.

For the isolation of VLDL ($d < 1.006$ g/mL), aliquots of supernatant S (3 mL) were overlaid with d 1.006 g/mL salt solution and centrifuged in the Type 40.3 rotor as before. On completion of ultracentrifugation, VLDL were removed in a volume of 1 mL, the next 1–2 mL was discarded, and the infranatant (II) was taken for isolation of LDL. After an increase in the density of infranatant II from 1.034 to 1.063 g/mL, aliquots (3 mL) were layered under a salt solution of the same density, and LDL was separated in the top 1 mL following centrifugation in the Type 40.3 rotor at 40 000 rpm for 18–20 h. Both VLDL and LDL were washed once by a single recentrifugation at their limiting density.

All salt solutions contained 0.02% sodium azide, 0.005% merthiolate, and 0.04% EDTA. The nonprotein solvent densities of these solutions were determined pycnometrically or with a digital precision density meter (Anton Paar, Graz, Austria; Model DMA 40) at 20 °C.

Lipoprotein fractions were exhaustively dialyzed at 4 °C against a solution containing 0.05 M NaCl, 0.04% EDTA, 0.02% sodium azide, and 0.005% merthiolate at pH 7.4.

(B) Density-Gradient Ultracentrifugation. Trout serum lipoproteins were also subfractionated on discontinuous salt density gradients according to the procedure of Redgrave et al. (1975). Gradients were constructed with the use of a Bu-

[2] This diet contained, on a weight percent basis, 33% whole ground wheat, 22% soya bean cake, 35% herring meal, 5% corn oil, 1% mineral mixture (Luquet, 1971), 2% vitamin complement (Halver, 1969), and 2% lignosulfite.

[3] The dialysate solution contained 0.04% EDTA (in addition to sodium azide and merthiolate), in order to protect the lipoproteins from any oxidative degradation which might be catalyzed by free metal cations.

chler Densiflow (Searle Analytic Inc.) in tubes of the Beckman SW 41 rotor; each gradient contained 4 mL of trout serum adjusted to a density of 1.21 g/mL with solid KBr (Radding and Steinberg, 1970). Centrifugation was performed in a Beckman L5-50 ultracentrifuge at 41 000 rpm (286 000*g*) for 24 h and at 6 °C. Control gradients made up entirely of salt solutions were ultracentrifuged simultaneously.

On completion of centrifugation, successive fractions of 1 mL were removed by aspiration from both control and serum-containing gradients. Lipoprotein fractions were dialyzed as indicated above.

The densities of fractions from control gradients were determined with the precision density meter at 20 °C.

Density Distribution of Serum Lipoproteins. For the purpose of the analytical ultracentrifugal analysis of the serum lipoprotein spectrum in the trout, lipoproteins of d <1.063 and of d <1.21 g/mL were isolated by preparative ultracentrifugation according to the general principles described in section A above. Analytical ultracentrifugal analysis was then performed by the modification of Mills et al. (1972) of DeLalla and Gofman's (1954) procedure.

Purity of Lipoprotein Fractions. Samples of each lipoprotein fraction prepared by sequential ultracentrifugation (i.e., VLDL and LDL) were subjected to immunodiffusion and immunoelectrophoresis against rabbit antisera to whole trout serum. Single (or double) precipitin lines of characteristic mobility (see Results) were revealed in each fraction after staining (separate slides) for protein and lipid. No lines were detected which were compatible with the presence of any contaminating serum proteins; such proteins were also absent from HDL preparations. The purity of fractions isolated by density gradient ultracentrifugation is considered separately under Results.

Characterisation of Lipoproteins. (1) Chemical Analysis. The separation and quantitation of the various lipid and protein components of each lipoprotein fraction were carried out according to the series of procedures described by Mills et al. (1977). Fatty acids were analyzed by gas-liquid chromatography of their methyl esters (Mills et al., 1977).

(2) Agarose Gel Electrophoresis. Electrophoresis in agarose gel was performed by the method of Noble (1968) using kits prepared by Bio-Rad.

(3) Electron Microscopy. Lipoprotein preparations were stained with 2% phosphotungstate (pH 7.4) and examined with a Philips EM 300 electron microscope, essentially as described by Forte et al. (1968). Details of our conditions have been given elsewhere (Chapman and Goldstein, 1976).

(4) Immunological Methods. Antisera to trout whole serum, LDL, and VLDL were prepared in rabbits of the Fauve de Bourgogne strain by procedures previously described (Goldstein and Chapman, 1976); the preparation of antisera to human serum LDL and VLDL was carried out as outlined earlier (Chapman et al., 1976). The trout preparations used as immunogens were whole serum (50 mg of protein), trout LDL (d 1.006–1.063 g/mL; 400 μg), and trout VLDL (d <1.006; 900 μg). Antiserum to human whole serum was obtained from the Institut Pasteur, Paris. Double immunodiffusion and immunoelectrophoresis were performed under conditions described earlier (Goldstein and Chapman, 1976).

Isolation and Characterization of Apolipoproteins. The total apoproteins of VLDL, LDL, and HDL were isolated by delipidation with organic solvents (Brown et al., 1969; Chapman et al., 1975). The residues were subsequently solubilized in sodium dodecyl sulfate containing buffers as described previously (Chapman et al., 1977b); aliquots (2–5 mg of protein) of apo-VLDL and apo-LDL were fractionated by gel-filtration chromatography on Sephadex G-200 (Herbert et al., 1973; Chapman et al., 1977b).

TABLE I: Distribution of Low-Density Lipoproteins in Trout Serum.[a]

species	LDL, S_f range 0–12	LDL, S_f range 12–20	VLDL, S_f range 20–100	VLDL, S_f range 100–400
trout	154.5 ± 59.3 (105.1–220.3)	31.8 ± 17.8 (11.4–44.1)	29.2 ± 19.2 (8.2–46.0)	0.47 ± 0.81 (0–1.4)
man	321	53	83	49

[a] Distributions were determined by analytical ultracentrifugation at a solvent density of 1.063 g/mL (Mills et al., 1977) and are expressed as mg/100 mL of serum. The values represent the mean ± SD of separate determinations on three different (random) sera; the ranges are given in parentheses. Data in man are taken from Mills and Taylaur (1971).

The apoprotein content of each lipoprotein fraction and the chromatographically isolated apoproteins were examined by the electrophoretic procedures outlined elsewhere (Chapman et al., 1975).

Amino acid analysis was performed on apoprotein samples (200–300 μg) after acid hydrolysis (Chapman et al., 1975) using a JEOL JLC 5AH amino acid analyzer equipped with an expanded range recorder.

Results

Serum Lipid and Lipoprotein Concentrations. In a series of six trout, the mean cholesterol concentration was 297 ± 97 mg/100 mL of serum (range 136–394), while that of triglyceride was 391 ± 192 mg/100 mL of serum (range 110–649). Triglyceride levels were thus variable, an observation reflected in the fivefold range seen in VLDL (S_f 20–400) concentrations (Table I); it is noteworthy that the predominant form of VLDL was of S_f 20–100. LDL levels varied less and were primarily present as S_f 0–12 substances (i.e., density 1.019–1.063 g/mL), rather than of greater S_f rate (i.e., S_f 12–20, d 1.006–1.019 g/mL).

A further three female fish were found to be hyperlipidemic; the mean cholesterol concentration was 500 mg/100 mL of serum (range 403–553) and the mean triglyceride level 1042 mg/100 mL of serum (range 884–1154). Analytical ultracentrifugal analysis of one of these sera revealed very high concentrations of both VLDL and LDL (VLDL, S_f 20–400, 673.4 mg/100 mL; LDL, S_f 0–20, 466.5 mg/100 mL). High concentrations of very low and low density lipoproteins did not appear to be correlated with vitellogenesis in females.

The principal lipoprotein in both male and female trout was always the HDL (d 1.063–1.21 g/mL), whose concentration was consistently greater than about 1500 mg/100 mL. Thus, HDL accounted for some 70% or more of the substances of d <1.21 g/mL.

Agarose Gel Electrophoresis. Electrophoresis in agarose gel of whole trout serum revealed up to six bands upon lipid staining (Figure 2); the majority of these bands migrated in the regions typical of α- and pre-β-human serum lipoproteins. Isolated VLDL electrophoresed as one or two diffuse bands of variable (between α and pre-β) mobility; these bands were located close to that of trout LDL, whose mobility was substantially greater than their human counterpart. Trout HDL were also of α mobility and were usually resolved as a broad diffuse band. Since the electrophoretic mobility of each trout serum lipoprotein class was not distinct from that of another, this technique could not be reliably employed as an aid in their

TABLE II: Mean Weight % Chemical Composition of Trout and Human Serum Lipoproteins.[a]

component	mean wt % composition					
	VLDL[b]		LDL		HDL	
	trout	man	trout	man	trout	man
cholesteryl ester	15.1	14.9	15.6	38.0	7.7	15.0
free cholesterol	6.9	6.4	6.7	9.0	3.4	2.9
triglyceride	41.9	49.9	26.9	11.2	15.5	8.0
phospholipid	26.5	18.6	27.1	22.1	26.5	22.7
protein	9.6	7.7	24.7	20.9	46.9	51.9

[a] Values presented are means of duplicate analyses of representative preparations. Data on the human fractions are taken from Mills and Taylaur (1971). [b] The densities of the lipoprotein fractions are VLDL < 1.006, LDL 1.006–1.063, and HDL 1.063–1.21 g/mL.

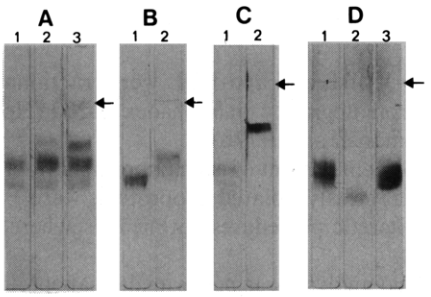

FIGURE 2: Agarose gel electrophoresis of trout serum lipoproteins. Electrophoresis was performed according to Noble (1968). Slides were stained for lipid with Sudan black. The sample wells are indicated by arrows. Samples are: slide A, 1, 2, and 3, different trout whole sera; slide B, 1, trout VLDL, 2 human VLDL; slide C, 1, trout LDL (*d* 1.006–1.063 g/mL); 2, human LDL (d 1.024–1.045 g/mL); slide D:1, 2, and 3, trout VLDL, LDL, and HDL, respectively.

identification, and immunoelectrophoresis was therefore used in its place.

Chemical Composition. The mean weight percentage composition of the three major classes of lipoproteins is presented in Table II and compared with representative data for man. The composition of trout VLDL approximated that of man, although the former were slightly richer in phospholipid and poorer in triglyceride. Both trout LDL and HDL differed more from their human counterparts, i.e., each was substantially poorer in cholesteryl ester and contained rather more triglyceride; the total proportions of neutral lipid (cholesteryl ester and triglyceride) were, however, similar in the LDL and in the HDL from the two species. Trout LDL exhibited high contents of polar components (phospholipid and protein) as compared to the human fraction.

It is noteworthy that cholesteryl esters were separated into two fractions (CE 1 and CE 2) by the thin-layer chromatographic system used (Mills et al., 1977). The former accounted for some 10.3, 10.1, and 5.0% of the total weight of VLDL, LDL, and HDL, respectively; CE 2 accounted for the remaining proportion of cholesteryl ester in each case (Table II). Wax esters normally migrate in the same position as CE 2 (Mills et al., 1977), but these substances could not be detected in trout lipoproteins.

The distribution of the fatty acids in the cholesteryl esters, triglycerides, and phospholipids of each lipoprotein class is shown in Table III. Of the two forms of cholesteryl ester, CE 2 was distinguished by its high proportion of long-chain acids, and particularly of 22:6, in each lipoprotein class; the phospholipids were also uniformly rich in 22:6. Of the saturated fatty acids, palmitic acid was typically the most abundant. Oleic acid was a prominent component of all the lipid esters with the exception of CE 2. The high degree of unsaturation of the lipid esters is notable, and less than 40% of the fatty acids

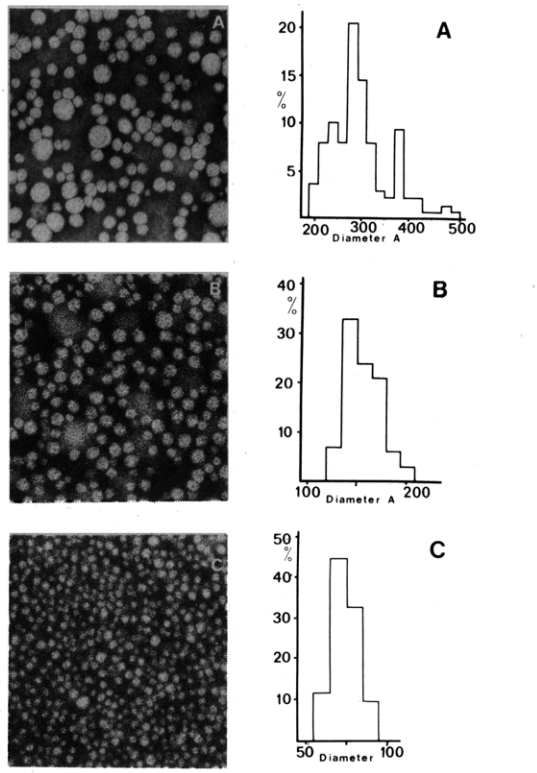

FIGURE 3: Electron micrographs of negatively stained trout serum lipoproteins and the distribution of particle diameters in each fraction. Samples are: A, VLDL; B, LDL of *d* 1.006–1.063 g/mL; C, HDL of *d* 1.063–1.21 g/mL. The final magnifications of the micrographs are 55 000× in A and 110 000× in B and C, respectively. The occasional particles (<3%) present at each extreme of the size ranges were excluded.

was saturated.

Electron Microscopic Studies. Electron microscopic examination of negatively stained trout VLDL showed them to range in diameter from about 200 to 500 Å (Figure 3). Free-standing particles tended to be spherical but deformed on contact. In the representative particle distribution shown in Figure 3A, the mean diameter was 287 Å and the mode 270 Å. The main group of particles ranged between 200 and 350 Å in diameter; a second smaller group, which was not always detectable, was of the order of 375 Å in size. Particles greater than 550 Å were seldom seen.

The LDL (*d* 1.006–1.063) was relatively homogeneous, the total range of diameters being 120–210 Å (Figure 3B). They were essentially spherical when free standing and displayed no obvious subunit structure. Their mean diameter was always less than 200 Å, and in the sample shown was 157 Å; the modal diameter was 142 Å. Trout LDL were substantially smaller than their human counterparts, which are typically of the order of 220 Å in mean diameter (Chapman and Goldstein, 1976).

TABLE III: Fatty Acid Composition of the Lipid Esters of Trout Serum Lipoproteins.[a]

	VLDL[b]				LDL				HDL			
fatty acid	CE(1)	CE(2)	TG	PL	CE(1)	CE(2)	TG	PL	CE(1)	CE(2)	TG	PL
14:0	0.6	0.8	1.8	1.2	0.6	0.7	1.5	1.2		0.5	1.6	1.0
16:0	25.7	7.0	16.6	25.0	30.5	4.8	17.2	25.7	28.7	4.2	17.2	24.8
16:1	4.3	2.4	9.0	3.5	4.0	2.3	8.9	3.3	4.3	2.1	8.9	2.9
18:0	6.5	3.1	3.5	11.5	8.5	2.5	3.6	11.6	7.5	1.8	3.5	10.5
18:1	28.5	3.3	41.9	14.1	25.2	2.5	42.8	14.6	30.0	2.8	40.7	13.3
18:2	9.4	0.8	10.1	8.3	8.2	1.3	10.4	8.7	9.6	3.2	10.3	8.7
18:3/20:1	12.7		5.8	2.3	11.4	0.8	5.2	2.5	12.8	0.5	4.8	3.0
20:2	5.9		2.7	2.1	5.3	1.0	1.6		4.9		2.0	2.3
20:4/22:1	4.1	5.5	1.9	1.6	4.0	5.9	0.7		2.3	6.0	1.4	2.4
20:5		6.7				6.9				6.5		
22:0	1.3			1.8	1.3	1.2				2.0		2.7
22:5												
22:6		65.5	6.9	23.7		64.5	8.2	25.6		66.9	9.7	28.6
24:0				4.9				7.1				
24:1												
Unident	1.0	5.0			1.2	5.8						

[a] The analyses were performed according to techniques described previously (Mills et al., 1977). Values are the weight % of fatty acids recovered. The analyses were made on lipoproteins isolated from a representative pool of serum, and values are the means of duplicate determinations: CE(1) and CE(2) represent the two forms of cholesteryl ester isolated by thin-layer chromatography; TG, triglyceride; PL, phospholipid. [b] The densities of the lipoprotein fractions are indicated in Table II.

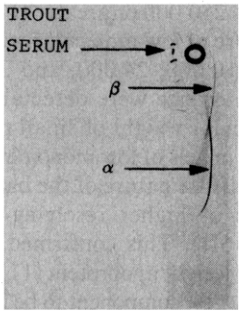

FIGURE 4: Detection of the principal trout lipoprotein classes by immunoelectrophoresis. Trout whole serum was reacted with its homologous antiserum and the slide stained for lipid with Sudan black.

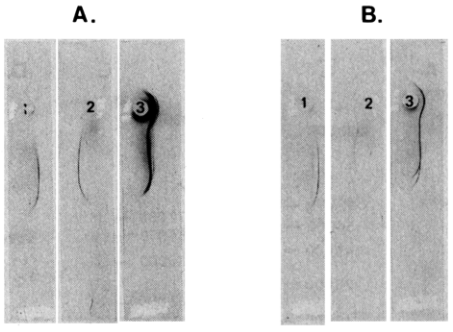

FIGURE 5: Immunoelectrophoretic patterns given by trout VLDL, LDL, and whole serum. Samples in A and B are: well 1, trout whole serum; well 2, trout LDL (*d* 1.006–1.063 g/mL); well 3, trout VLDL. A, antiserum to trout LDL (*d* 1.006–1.063 g/mL); B, antiserum to trout VLDL, respectively, in central troughs.

Only a few particles in this LDL fraction approached the diameter of the smallest VLDL (<3%), indicating little overlap in their particle contents.

The HDL (*d* 1.063–1.21 g/mL) fraction consisted of very small particles (Figure 3C), 97% of which ranged in diameter from 60 to 110 Å; no subunit structure could be discerned. The mean and modal diameters were 77 and 71 Å, respectively. The distribution of sizes in the LDL and HDL suggested that only a slight degree of overlap existed in their particle contents.

Immunological Studies. Immunoelectrophoresis of trout whole serum against its homologous antiserum (Figure 4) indicated the presence of two principal lipoprotein classes, one corresponding to that of β and one to that of α mobility. These precipitin lines presented a reaction of nonidentity. An identical slide stained for protein showed the presence of serum immunoglobulins, β_2- and β_1-globulins, α_2- and α_1-globulins, and albumin. The mobility of one of the two precipitin lines in trout whole serum which stained for both lipid and protein corresponded to that of the β-globulins, while the other migrated in a position similar to that of the α_1-globulin.

The electrophoretic mobility of the two lipoproteins differed somewhat from that of the corresponding fractions of human whole serum when reacting with its homologous antiserum; thus, the β and α migrating lipoproteins of the trout showed a faster and a slower mobility, respectively, as compared to their human counterparts.

Trout whole serum, VLDL and LDL (*d* 1.006–1.063 g/mL), were studied with antisera to trout VLDL and to trout LDL by immunodiffusion and immunoelectrophoresis. Upon immunoelectrophoresis, LDL and trout whole serum gave a single precipitin line with antiserum to LDL (Figure 5A), whereas a double arc was produced by VLDL. In reacting with an antiserum to trout VLDL, all three preparations, i.e., serum, VLDL, and LDL, presented two precipitation lines (Figure 5B). These findings suggest that two antigenic determinants are present in both LDL and VLDL; they are, however, more strongly represented in VLDL than in LDL, since LDL elicits antibodies to both determinants (VLDL reacted with antiserum to LDL to give two lines) and itself reacts to produce two precipitation arcs in the presence of a strong antiserum such as anti-VLDL.

Trout VLDL and LDL were compared upon immunodiffusion and immunoelectrophoresis with the corresponding human fractions. The trout fractions failed to react with antisera to human whole serum, to human VLDL, and to human LDL, irrespective of the amount of antigen employed. On the contrary, human LDL and particularly human VLDL reacted with large amounts of antisera to trout whole serum and to trout VLDL and LDL; these reactions necessitated two- to threefold larger amounts of trout antisera than the reactions with the homologous antisera.

TABLE IV: Amino Acid Composition of Trout Serum VLDL, LDL, and HDL.[a]

amino acid	apo-VLDL (2)	apo-LDL (4)	apo-HDL (4)
Lys	7.0	7.4 ± 0.18	9.2 ± 0.38
His	1.4	1.7 ± 0.10	1.4 ± 0.17
Arg	3.0	2.8 ± 0.08	4.0 ± 0.17
Asp	13.1	11.2 ± 0.40	7.4 ± 0.49
Thr	6.9	7.1 ± 0.23	4.6 ± 0.33
Ser	6.2	6.7 ± 0.32	4.3 ± 0.38
Glu	15.2	12.3 ± 0.52	18.3 ± 1.71
Pro	3.6	3.8 ± 0.24	3.8 ± 0.37
Gly	5.2	7.7 ± 1.01	5.5 ± 0.33
Ala	9.8	9.8 ± 0.41	12.5 ± 0.05
Val	5.2	5.8 ± 0.10	6.3 ± 0.15
Met	1.4	1.4 ± 0.64	1.7 ± 0.06
Ile	4.8	5.4 ± 0.14	3.4 ± 0.06
Leu	10.9	10.9 ± 0.43	11.1 ± 0.30
Tyr	2.8	2.4 ± 0.18	4.4 ± 0.64
Phe	3.5	3.5 ± 0.06	1.9 ± 0.00

[a] Values are the means ± SD of the number of samples given in parentheses; the hydrolysate of each sample was analyzed in duplicate. Values are expressed as mol of each amino acid/100 mol of amino acid residues. The densities of the lipoprotein fractions are as indicated in Table II.

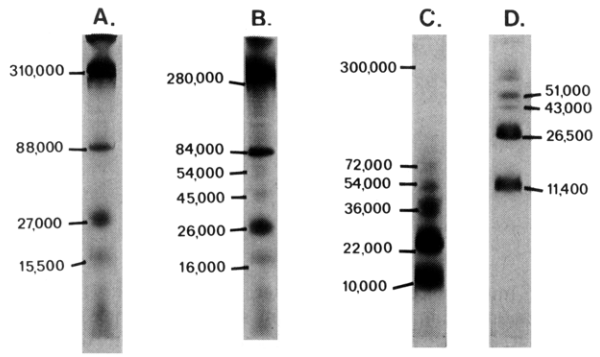

FIGURE 6: Electrophoretic patterns in sodium dodecyl sulfate–polyacrylamide gel of trout apo-VLDL, apo-LDL (d 1.006–1.063 g/mL), and apo-HDL. Gels were stained with Coomassie brilliant blue; molecular weights were calculated from a series of purified marker proteins. Samples are (A) apo-VLDL, (B) apo-LDL, and (C) apo-HDL and were electrophoresed in 3.3% monomer gels; sample (D) apo-HDL was run in a 7.5% gel.

Characterization of the Apoprotein Components. Examination of the total amino acid compositions of apo-VLDL, apo-LDL, and apo-HDL suggested that HDL differed from VLDL and LDL in its apoprotein composition (Table IV). Thus, apo-HDL exhibited the highest proportions of lysine, glutamic acid, and alanine; the lowest proportion of aspartic acid; and an inverse ratio (2.3:1.0) of tyrosine to phenylalanine.

This suggestion was confirmed upon examination of the apoprotein components of the three lipoprotein classes by electrophoretic techniques. Examination of the total apoproteins of VLDL, LDL, and HDL in sodium dodecyl sulfate–polyacrylamide gel of low monomer concentration (3.3%) typically revealed that apo-VLDL and apo-LDL were qualitatively indistinguishable (Figure 6). Thus, the predominant component of each was represented by a diffuse intense band of high molecular weight (>250 000); such behavior is typical of an apolipoprotein resembling apo-B in man. Intense bands with molecular weights of 84 000–88 000, and 26 000–27 000 were also evident. In addition, a number of fainter bands with

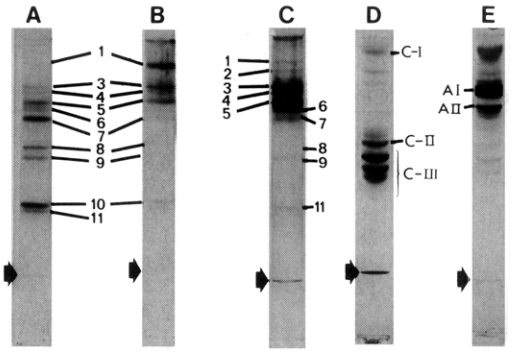

FIGURE 7: Electrophoretic patterns of tetramethylurea-soluble apoproteins of trout and human serum lipoproteins in basic polyacrylamide disc gel. Samples of the tetramethylurea-soluble extracts were electrophoresed in 7.5% monomer gels containing 8 M urea at pH 8.91; gels were stained with Coomassie brilliant blue. Samples are (A) trout VLDL, (B) trout LDL (d 1.006–1.063 g/mL), (C) trout HDL, (D) human VLDL, and (E) human HDL. Apolipoproteins are numbered in descending order (see Table V). The arrows indicate the dye front in each gel.

molecular weights of about 54 000, 45 000, 16 000 and 10 000, could be identified.

Apo-HDL was distinct in exhibiting only a trace of material of molecular weights 250 000 or greater (Figure 6C). Its most prominent bands were of low molecular weight and in particular were those of 10 000, 24 000, and 36 000. Additional components of greater size were detectable. Since the estimation of the molecular weight of small proteins (<50 000) is fraught with error in gels of low monomer concentration and is worsened by the diffuse nature of the bands, apo-HDL was reexamined in gels of higher resolving power (i.e., 7.5% monomer) (Figure 6D). This confirmed the presence of a major low molecular weight apoprotein (11 400) and estimated the size of the next largest component to be 26 500; components of 43 000, 51 000, and >70 000 (multiple) were also in evidence. It is plausible that the bands of 16 000, 26 000, 45 000, and 54 000 identified in apo-VLDL and apo-LDL correspond to those of 11 400, 26 500, 43 000, and 51 000, respectively, in apo-HDL.

Electrophoresis of the tetramethylurea-soluble apoproteins in alkaline polyacrylamide gel containing urea showed the presence of several components in each lipoprotein class (Figure 7); in order to permit comparison between gels, the relative electrophoretic mobilities[4] of each band were calculated and this index was employed in their identification (Figure 7). Numerous bands of rapid mobility (bands 8–11, electrophoretic mobilities 0.45–0.72) were visible in the tetramethylurea-soluble fraction of VLDL; band 10 was particularly intense. This band and those adjacent to it were poorly represented in LDL and HDL. On the contrary, the major bands in HDL (3–5, with mobilities of 0.21, 0.23, and 0.27, respectively) were less pronounced in LDL and still less in VLDL. LDL was unique in that its major tetramethylurea-soluble component was band 1, of which there were only traces in VLDL and HDL.

Comparison of these electrophoretic patterns with those typical of man (Figure 7D,E) indicated the greatest degree of similarity to exist between human and trout HDL, in which the two major apoproteins (bands 3 and 4 in trout and AI and AII in man) were indistinguishable in mobility: trout HDL gave the same pattern under both reducing and nonreducing conditions. The predominance of the high- and low-mobility

[4] Electrophoretic mobility is defined as the ratio of the distance of migration of the individual apolipoprotein to that of the dye front.

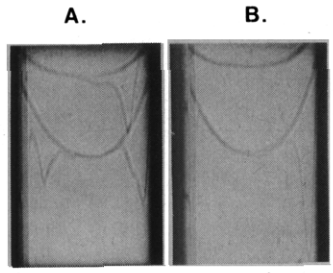

FIGURE 8: Distribution of lipoproteins in (A) human serum and (B) trout serum during analytical ultracentrifugation at 52 640 rpm at 26 °C. The upper patterns in A and B show distributions obtained after 56 min in NaCl solution of density 1.063 g/mL, while the lower profiles were obtained in an NaCl/KBr solution of density 1.21 g/mL under the same conditions.

apoproteins typically seen in human VLDL (i.e., C-II and C-III on the one hand and C-I on the other) was not, however, evident in trout VLDL.

Density Distribution of LDL. As noted above, the electrophoretic pattern given by apo-HDL (*d* 1.063–1.21 g/mL) in dodecyl sulfate–polyacrylamide gel indicated the presence of small amounts of an apolipoprotein B like component in the lipoproteins of *d* >1.063 g/mL. This possibility was examined further by analytical ultracentrifugal analysis of trout serum lipoproteins at solvent densities of 1.063 and 1.21 g/mL and by comparison of their respective density distributions with those of the human substances (Figure 8). It may be clearly seen that the complete distribution of low-density substances in human serum may be detected at a solvent density of 1.063 g/mL; in contrast, only very small amounts of such substances were detectable under similar conditions in the trout (Figure 8B, upper section). The complete distribution of trout LDL is, however, completely resolved at a density of 1.21 g/mL (Figure 8B, lower section); its proximity to the peak of HDL is notable. In contrast, the peak of low-density substances is well separated from that of high-density in human serum under the same conditions (Figure 8A, lower section). Further analysis of the flotation rates of trout LDL indicated that this fraction could be completely isolated by use of a higher limiting density of approximately 1.080 g/mL (i.e., instead of 1.063 g/mL).

This observation was confirmed by fractionation of trout serum lipoproteins on a discontinuous density gradient. Fractions I–VI, corresponding to densities of <1.008, 1.008–1.025, 1.025–1.054, 1.054–1.078, 1.078–1.106, and 1.106–1.138 g/mL, respectively, were removed by aspiration. Immunological analysis of these fractions by immunoelectrophoresis employing antiserum to trout whole serum revealed that lipoproteins of pre-β and β mobility were present up to and including fraction IV (density limit 1.078 g/mL); furthermore, fractions I–IV contained exclusively lipoproteins exhibiting β reactivity. In contrast, fractions below IV (i.e., *d* >1.078 g/mL) exhibited only lipoproteins of α mobility upon immunoelectrophoresis, and those of β mobility could not be detected. These observations clearly indicate that β lipoproteins in the trout extend to a density of 1.078 g/mL. Fraction IV (*d* 1.054–1.078 g/mL) lipoproteins comprised some 60% of the total substances of *d* <1.078 g/mL, while those of *d* <1.008, 1.008–1.025, and 1.025–1.054 g/mL accounted for 7.2, 4.0, and 27.7%, respectively.

Electron microscopic studies of the gradient fractions served to confirm and extend the data obtained on VLDL (*d* <1.006) and LDL (*d* 1.006–1.063 g/mL) fractions isolated by sequential ultracentrifugation (data not shown). It is noteworthy that the mean diameter of fraction III (*d* 1.025–1.054 g/mL) was 187 Å, while that of fraction IV (*d* 1.054–1.078 g/mL) was 110 Å, thus indicating the considerable size heterogeneity of β-lipoproteins in trout.

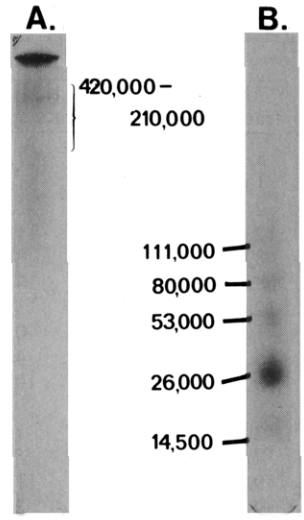

FIGURE 9: Electrophoretic patterns in sodium dodecyl sulfate–polyacrylamide gel of column fractions I and II following gel chromatography. Patterns: (A) fraction I from trout apo-LDL-2 (*d* 1.025–1.078 g/mL) and (B) fraction II isolated from the same preparation of trout apo-LDL-2 as that in A. The monomer concentration of the gels was 3.3%; molecular weights were determined from a series of purified marker proteins.

The β-reacting lipoproteins isolated in the density interval 1.025–1.078 g/mL on density gradients will be referred to as LDL-2 on the basis of their β reactivity and content of an apolipoprotein B-like component (see next section); in strict terms, however, this fraction is not of "low density". We have used this nomenclature to differentiate the *d* 1.025–1.078 substances from the low-density (β-reacting) lipoprotein fraction, i.e., LDL, isolated by preparative ultracentrifugation in the classical density range 1.006–1.063 g/mL.

Fractionation and Characterization of the Major Apolipoproteins of VLDL and LDL. The total apoproteins of VLDL (*d* <1.006 g/mL) and of LDL-2 (*d* 1.025–1.078 g/mL, i.e., fractions III and IV from the gradient subfractionation) were solubilized in a sodium phosphate (0.01 M) buffer containing 1% sodium dodecyl sulfate and 1% β-mercaptoethanol at pH 8.0 and chromatographed on Sephadex G-200. Two peaks were obtained from both apo-VLDL and apo-LDL-2; only their relative areas differed. Elution of the first peak was coincident with the exclusion volume, thus indicating its high molecular weight (>250 000). Quantitation of the protein recovered in the two fractions indicated fraction I to represent some 35% of apo-VLDL and some 60% of apo-LDL-2. The high molecular weight nature of fraction I from both apo-VLDL and apo-LDL-2 was confirmed by electrophoresis in dodecyl sulfate–polyacrylamide gel (Figure 9); the major zone of stained material was diffuse and corresponded to a range in molecular weight from 210 000 to 420 000. Some faintly stained regions could also be recognized and these ranged in size from about 100 000 to 210 000. Thus, the physical properties of the high-molecular-weight fraction I resembled those typical of human apolipoprotein B. Further resemblance to the human apolipoprotein was seen in the amino acid composition of fraction I from trout apo-VLDL and apo-LDL-2 (Table V), although some differences were evident. Thus, trout fraction I appeared richer in aspartic and glutamic acids, alanine, and valine but rather poorer in lysine, proline, leucine, and phenylalanine.

Fraction II, isolated both from apo-VLDL and apo-LDL-2, was distinct in its amino acid composition from fraction I in

TABLE V: Amino Acid Compositions of Subfractions of Apo-VLDL and Apo-LDL from Trout Serum.[a]

	trout						
amino acid	VLDL fraction I (3)	LDL-2 fraction I (2)	VLDL fraction II (3)	LDL-2 fraction II (2)	human apo-B	shark apo-B	hagfish apo-B
Lys	5.7 ± 0.10	7.3	6.7 ± 0.60	8.3	8.0	9.2	6.8
His	1.6 ± 0.15	1.2	1.1 ± 0.06	1.1	2.2	2.4	2.0
Arg	2.8 ± 0.15	2.8	2.9 ± 0.34	3.9	3.3	4.2	4.0
Asp	12.4 ± 0.61	11.6	10.2 ± 0.28	6.6	10.9	11.5	13.2
Thr	8.0 ± 0.12	8.4	7.1 ± 0.17	5.0	6.7	7.3	5.5
Ser	8.5 ± 0.25	6.4	7.0 ± 0.13	4.9	8.7	9.5	9.9
Glu	12.6 ± 0.20	13.6	19.4 ± 0.90	20.3	10.8	11.5	11.9
Pro	1.0 ± 0.06	0.9	1.0 ± 0.13	5.9	4.2	3.0	4.0
Gly	5.5 ± 0.40	4.7	5.0 ± 0.26	5.0	5.5	7.0	6.6
Ala	9.2 ± 0.29	10.8	11.8 ± 0.53	11.1	7.0	6.6	6.9
Val	9.4 ± 0.47	9.5	7.2 ± 0.38	6.6	5.6	5.3	4.1
Met	2.7 ± 0.20	1.4	2.3 ± 0.12	1.9	1.8	0.9	3.0
Ile	4.9 ± 0.32	5.0	3.2 ± 0.17	4.2	5.0	5.3	3.9
Leu	9.2 ± 0.12	11.2	9.1 ± 0.50	11.1	12.3	9.7	10.3
Tyr	2.6 ± 0.21	2.3	4.0 ± 0.14	2.0	3.3	2.8	3.1
Phe	2.8 ± 0.44	2.9	1.9 ± 0.17	2.1	4.9	3.8	4.8

[a] The total apoproteins of trout serum VLDL (d <1.006 g/mL) and LDL-2 (d 1.024–1.078 g/mL) were fractionated by gel-filtration chromatography on Sephadex G-200 (see Materials and Methods). Values are expressed as mol of each amino acid/100 mol of amino acid residues and represent the means ± SD of the number of preparations given in parentheses; the hydrolysate of each preparation was analyzed in duplicate. For comparative purposes, the data of Gotto et al. (1972) on the composition of human apolipoprotein B and those of Goldstein et al. (1977) and of Mills et al. (1977) on the composition of the apolipoprotein B like components from the hagfish (*Myxine glutinosa*) and shark (*Centrophorus squamosus*), respectively, are presented.

each case (Table V). In particular, it was enriched in glutamic acid (ca. 20%) and alanine; some differences (notably in aspartic acid, proline, and tyrosine contents) were, however, seen in the compositions of fraction II from apo-VLDL and from apo-LDL-2. Such dissimilarities are consistent with the differing complement of tetramethylurea-soluble components seen in these two apoproteins (Figure 7). Examination of fraction II isolated from apo-LDL-2 by dodecyl sulfate-polyacrylamide gel electrophoresis (Figure 9) showed it to present five bands, whose molecular weights were 14 500, 26 000, 53 000, 80 000, and 110 000, respectively. The most intense band was that of 26 000; that of 110 000 was present in trace amounts.

Discussion

In the present studies, we have noted the existence of some remarkable similarities between the physical and chemical properties of the principal serum lipoproteins (VLDL, LDL, LDL-2, and HDL) of the rainbow trout and those of the corresponding human fractions, notwithstanding the large evolutionary distance between these species. Furthermore, our data concur with those of Nelson and Shore (1974) on the HDL of pink salmon and of Mills et al. (1977) on the serum lipoproteins of the shark *Centorphorus squamosus*, since these authors have also noted a high degree of analogy between the corresponding fish and human substances.

In contrast to the distinction seen in the chemical and physical properties of trout and human LDL (d 1.006–1.063 g/mL), the chemical compositions of trout and human VLDL were alike (Table II), although the proportion of polar components (phospholipid and protein) was greater in trout (36.1%) than that typically seen in man (26.1%). If the general structure of trout and human VLDL is similar [i.e., in man the polar components are believed to form a surface coat within which are the neutral lipid components (Sata et al., 1972)], then these data predict that trout VLDL should be smaller in diameter than those typically encountered in man (Sata et al., 1972). This was in fact the case, electron microscopic studies showing the majority of trout VLDL to be less than 500 Å in diameter, while those of man are usually more heterogeneous and larger (Quarfordt et al., 1972); despite this distinction, however, the morphology of trout VLDL was indistinguishable from that of the human substances. Similarly small VLDL particles have also been observed in the hagfish (*Myxine glutinosa*) (Chapman and Mills, unpublished observations) and, in addition, in the chicken (Chapman et al., 1977b) and probably reflect basic differences between the absorption and transport of exogenous lipids in these species and that in mammals. In this regard, it is noteworthy that some doubt exists as to the presence of a mesenteric lymphatic circulation in trout, as well as to its ability to secrete chylomicron-like particles from the enterocytes (Robinson and Mead, 1973). Bergot and Flechon (1970a,b) have, however, observed lymphatic vessels in the basal portions of the intestinal wall and have reported the presence of chylomicron-like particles (<2000 Å) in enterocytes as well as in the portal and lymphatic systems. Accordingly, these authors have suggested that such particles may be secreted into both the portal (as in chicken) and lymphatic circulations. We have recently confirmed the existence of small chylomicrons (diameter 800–2000 Å) in trout serum by negative-stain electron microscopy (Chapman, M. J., and Fremont, L., unpublished observations).

In view of the phylogenetic distance between *S. gairdnerii* and man, it was of considerable interest to compare the major apolipoproteins of this teleost with their human counterparts. In addition, since all classes of trout serum lipoproteins were distinct from their human counterparts in containing particularly large proportions (>60%) of unsaturated fatty acids in their lipid esters, it may reasonably be asked whether their respective apoprotein components resemble those of man or whether they have been modified to accommodate the high degree of unsaturation of the lipids; such a modification could also serve to complement the ability of the lipids to maintain the hydrophobic cores of the lipoproteins (VLDL, Sata et al., 1972; LDL, Deckelbaum et al., 1975; HDL, Scanu, 1972) in a liquid phase(s) at the low temperatures encountered by the animal. Despite the differing nature of their lipid moieties, however, the major protein component(s) of each trout lipoprotein class, and particularly in VLDL and LDL, bore a marked resemblence to its counterpart(s) in the corresponding

human lipoprotein fraction.

Thus, the major protein component of trout VLDL, LDL (d 1.006–1.063 g/mL), and LDL-2 (d 1.025–1.078 g/mL) and representing some 60% of apo-VLDL and apo-LDL-2, respectively, was a protein whose physical and chemical properties resembled those typical of human apolipoprotein B; henceforth, we propose to refer to this component as trout apolipoprotein B. Some dissimilitude between human and trout apo-B was, however, evident and this primarily concerned their amino acid compositions. Our immunological studies also suggest that the antigenicity of apo-B in trout VLDL and LDL is distinct from that in the human fractions; however, it must be borne in mind that both trout apo-VLDL and apo-LDL contain substantial proportions of protein components other than apo-B, and these components could account, at least in part, for the differing reactivities of the parent particles. It is particularly noteworthy that in earlier studies we have isolated an apolipoprotein B like component from the serum LDL of a shark (Mills et al., 1977) and from that of a cyclostome, the hagfish *Myxine glutinosa* (Goldstein et al., 1977). These data, considered together with those presented here, indicate that analogues of human apolipoprotein B occur ubiquitously in vertebrates, a finding consistent with the suggestion that apo-B is a fundamental lipid-transporting protein, at least a part of whose structure has been highly conserved during vertebrate evolution.

The high-density lipoproteins of trout serum possessed two major protein components, whose molecular weights were in the ranges 22 000–26 500 and 10 000–11 400, respectively (Figure 6). These polypeptides exhibited similar mobilities to those of human apolipoproteins A-I and A-II upon electrophoresis in basic polyacrylamide gel (Figure 7) and are denoted as bands 3 and 5; Skinner and Rogie (1977) have also reported the presence of such components in trout HDL. It is noteworthy, however, that the mobility of the band 5 polypeptide was unaltered by reducing agents, suggesting that it is distinct from its human counterpart, apo-A-II, in lacking a disulfide bond. In view of the resemblance between the electrophoretic mobility and molecular weight (ca. 24 000) of band 3 and those of human apo-A-I (mol wt ca. 28 000; Baker et al., 1973) and of the parallel similarities between band 5 (mol wt ca. 10 500 under reducing conditions) and human apo-AII (monomer mol wt 8700; Brewer et al., 1972), it appears that trout HDL may possess counterparts to the A apolipoproteins of man. In salmon, Nelson and Shore (1974) found considerable analogy between the two major HDL apolipoproteins and those of man, although the amino acid compositions of corresponding components in the two species differed markedly.

The minor, low-molecular-weight protein components of each trout lipoprotein class tended to be distinct from those typically seen in the equivalent human fraction, at least as judged by electrophoretic methods based on charge and on size. Some components (bands 8–11, Figure 7), with mobilities resembling those of human apolipoproteins C-II and C-III, were, however, detectable; in this context, it is noteworthy that trout VLDL could activate a purified bovine milk lipoprotein lipase (M. C. Glangeaud, personal communication), suggesting the presence of a counterpart to apo-CII in their protein moiety. Of the β-migrating lipoproteins, the VLDL contained both the largest proportion (some 65% of the total protein moiety) and number (about 11) of low molecular weight components; Kane et al. (1975) have reported the presence of a slightly lower content (56%) of soluble apolipoproteins in a human VLDL fraction with a similar mean diameter (367 Å) to that of trout VLDL (287 Å) in the present study.

The concentrations of HDL (>1500 mg/100 mL of serum) in trout were markedly elevated and substantially higher than those seen in any of the 18 animals examined by Mills and Taylaur (1971) (with the exception of the pigeon). They are, however, similar to those observed in another Salmonid, the pink salmon (*Oncorhynchus gorbuscha*), whose HDL levels were of the order of 3000 mg/100 mL of serum [calculated from Nelson and Shore (1974)], but differ from those of juvenile sockeye salmon (*O. nerka*), which were rather lower (238 mg/100 mL of serum) (Reichert and Malins, 1974).

The pacific sardine also appears to contain moderately large amounts of high-density substances (ca. 1000 mg/100 mL of serum) (Lee and Puppione, 1972). Such high HDL concentrations do not appear to be typical of fish, however, since Mills and co-workers (Mills et al., 1977; Mills and Taylaur, 1977) have noted relatively low HDL levels (often amounting to <10% of the total lipoproteins of d <1.21 g/mL) in several species of shark and sturgeon; similar observations have also been made in the spiny dogfish (Lauter et al., 1968) and dogfish (Mills and Taylaur, 1971).

In the late prespawning pink salmon, Nelson and Shore (1974) suggested that residual fat transport was effected by the HDL in the absence of significant amounts of VLDL and LDL. This hypothesis would not appear to explain fully the high HDL levels found in the present study, since they were consistently detected in fish with moderately high LDL concentrations and with measurable amounts of VLDL. Moreover, our trout were not in a prespawning state. Trout was also distinct from the salmon in that cholesteryl esters represented only 14% of the HDL lipids, while in salmon this figure was 30%, a value typical of human HDL. It is noteworthy, however, that the total proportions of "core" lipid (i.e., triglyceride and cholesteryl ester) in trout, salmon, and human HDL were essentially indistinguishable (some 42, 44, and 47%, respectively). Whether the above differences between trout HDL and those of salmon and man emanate from fundamental dissimilarities in the metabolism and transport of neutral lipids in these species remains indeterminate, and further metabolic studies are required to establish this point.

In conclusion, the present investigations demonstrate that the VLDL and LDL of trout have some physicochemical properties in common with their human counterparts, the most notable being that their major protein component is analogous to human apolipoprotein B. The most significant dissimilarity concerns the distribution of the low-density lipoproteins, of β-reactivity upon immunoelectrophoresis, which extend to a higher density in trout (1.078 g/mL) than in man (1.063 g/mL). Trout HDL also shared some characteristics typical of the human substances, including their possession of polypeptides which may be related to the human AI and AII apolipoproteins. All classes of trout lipoproteins were distinct from those of man in their complement of tetramethylurea-soluble protein components (excluding the major HDL proteins) and in the high proportions of unsaturated fatty acids in their lipid esters. Despite such dissimilarities, however, our data suggest that the basic molecular organization of trout lipoproteins does not differ substantially from that characteristic of the human substances, i.e., a largely hydrophobic core containing the neutral lipids (cholesteryl ester and triglyceride) and a hydrophilic coat of the more polar substituents (particularly protein and phospholipid).

Acknowledgments

We gratefully acknowledge Dr. M. H. Laudat for her support. We express gratitude to Dr. J. Breton-Gorius, to Professor J. Rosa, and to Dr. Y. Beuzard for the use of laboratory fa-

cilities. Y. Blouquit provided invaluable assistance in amino acid analysis.

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