review

Plasma lipoproteins in fish

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Various strategies have been developed by metazoans for the transport of exogenous or endogenous lipids towards different tissues, where they are stored or oxidized. In all cases, this transport involves macromolecular protein-containing complexes: lipoproteins. For example, in direct relation with their open circulatory system and the differentiation of a lipid storage tissue, the fat body, insects have developed a system built around the lipoprotein lipophorin, which circulates in the hemolymph, shuttling between the fat body cells producing it and the parenchymal cells to which it has direct access (1).

A complex system has evolved in vertebrates in the context of a closed circulatory system, at the same time as the capacity of storing triglycerides in adipose tissue. As a result of an adequate assembling of different constituents in the blood, there are several lipoprotein classes that assure lipid transport. They can be classified according to hydrated density, ultracentrifugation flotation velocity (S_f), electrophoretic mobility on agarose or, more recently, apolipoprotein composition. Different classes are thus distinguished by order of density and decreasing size: chylomicrons, VLDL (very low density lipoproteins), IDL (intermediate density lipoproteins), LDL (low density lipoproteins) and HDL (high density lipoproteins). The same class of lipoproteins defined by its density may be composed of particles with different apolipoprotein compositions, thereby determining a different metabolic destiny. In the plasma, each lipoprotein is subjected to permanent changes by interactions with tissues and also with particles of different sizes and origins, which may or may not result in enzymatic transfers of lipids or apolipoproteins.

From a functional standpoint, mammalian plasma lipoproteins are integrated into two transport systems, exogenous and endogenous. In the former, lipoproteins (chylomicrons, VLDL) are large particles with low densities. They are vectors for large quantities of nonpolar lipids, primarily triglycerides. They transport long-chain fatty acids to the well-developed adipose tissue, where they will be stored, or to other tissues where they will be oxidized. Since the circulatory system is closed, a preliminary step of triglyceride hydrolysis by a lipoprotein lipase (LPL) attached to the cell membrane of endothelial cells precedes the transfer of fatty acids to cells which will utilize them. This exogenous system is also used to transfer dietary cholesterol to liver cells which internalize the particles resulting from this incomplete hydrolysis (remnant chylomicrons, remnant VLDL, LDL). The second (endogenous) system originates in adipose tissue and the liver. The use of triglycerides stored in adipose cells requires their hydrolysis by a hormone-dependent triglyceride lipase and the transport of released fatty acids as complexes with a carrier protein, albumin, while the liver controls circulating lipid levels by synthesizing and secreting various lipoproteins.

The study of lipid transport systems in fish (20,000 species), located at the base of the vertebrate evolutionary ladder, is a very exciting field from a phylogenetic standpoint. However, it should be remembered that the term "fish" covers a heterogeneous and complex set of organisms and that the phylogenetic relationships among the seven classes of vertebrates (**Fig. 1C**) remain hypothetical for lower vertebrates. The vertebrates are separated into two subbranches, the Agnatha and Gnathostomes, jawless and jawed fish. Hagfish, usually grouped with lampreys to constitute the Cyclostomes, the only living forms of Agnatha, differ from jawed and jawless fish by such a large number of characters that there is a current trend to consider them as a separate group, a close relative of Agnatha and Gnathostomes. We will thus discuss only the lampreys, aquatic and pisciform, which we consider as jawless fish. Fish in the strict sense, the Gnathostomes or jawed fish, are in turn divided into two classes, the Chondrichthyes and the Osteichthyes (Fig. 1C). The Chondrichthyes are a special evolutionary line in that they have no descendants among the land vertebrates; the latter have all

Abbreviations: apo, apolipoprotein; VHDL, HDL, LDL, IDL, and VLDL, very high density, high density, low density, intermediate density, and very low density lipoproteins, respectively; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; LPL, lipoprotein lipase; HPLC, high performance liquid chromatography.

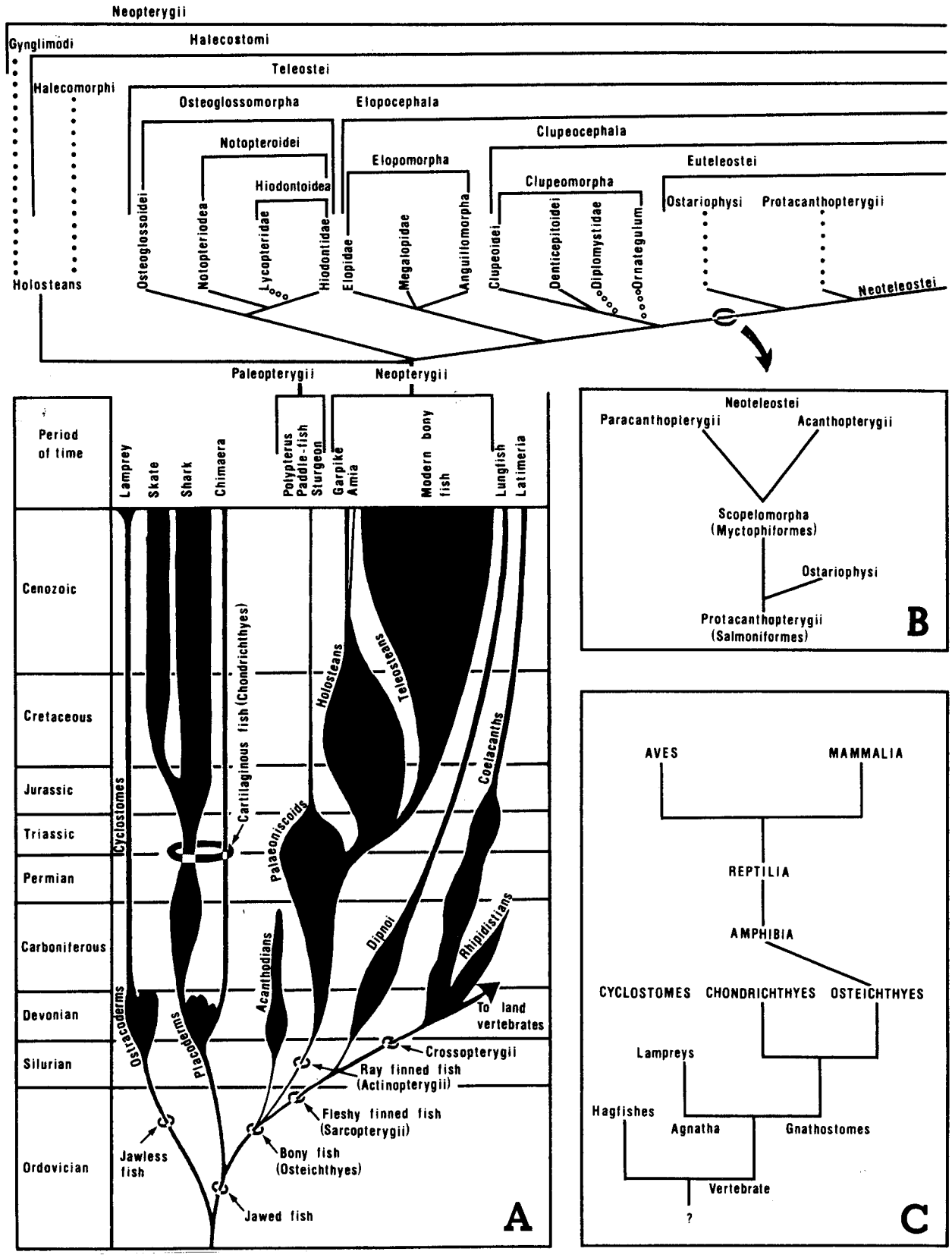

Fig. 1. Diagrams showing the evolutionary relationships between and within the different groups of fish (A and B) and among the seven classes of vertebrates (C). Summary taken from references 273–277; (ooooo) fossil forms.

arisen from certain Osteichthyes (Crossopterygii) (Fig. 1A). The most numerous data on fish plasma lipoproteins (for prior reviews see 2–4) concern the most recent Actinopterygii (see Table 3), teleost fish and among them the Salmoniformes (or Protacanthopterygii), considered as primitive Euteleostei (Fig. 1A). Some authors have even admitted a direct lineage between the Salmoniformes and the three groups arising from them at different levels: on the one hand Ostariophysi, and on the other Paracanthopterygii and Acanthopterygii, constituents of the most recent Teleostei (Neoteleostei) (Fig. 1B).

Fish are poikilothermic vertebrates that preferentially use lipids rather than carbohydrates as an energy source (5, 6), in the context of a perfectly closed circulatory system, independent of interstitial lymph. Lipids in the form of triglycerides and wax esters can be stored in various tissues, in particular muscle and liver (see ref. 7 for review), since true adipose tissue has developed in only several rare species (7–9). In addition, fish are generally oviparous and their eggs are rich in protein and lipid yolk reserves. A special lipoprotein, vitellogenin, secreted by liver cells plays a fundamental role in the process of vitellogenesis.

A. Plasma lipids and lipoproteins

Except for the Chondrichthyes (skates, sharks, etc.) and Osteichthyes Ginglymodi (garpike), the use of standards, applied to mammals and man in particular (10–12), classifies fish as hyperlipidemic and hypercholesterolemic (**Table 1** and **Table 2**). For example, in fed rainbow troutlets (13), the plasma transports three times more lipids (1940 mg/dl vs. 685 mg/dl) and cholesterol (303 mg/dl vs. 106 mg/dl) than fed rats (14). The values may even be much higher, e.g., cholesterolemia in this species can reach values 12 times higher than rats (Table 2). Most of this cholesterol is present in an esterified form in the plasma of most fish species (Table 1). In fish, lipids are integrated into lipoproteins in the proportion of at least 85% in the spiny dogfish (15), and 93% and 95% in mature male rainbow trout (16) and channel catfish (17), respectively. Thus, Agnatha and Osteichthyes generally are hyperlipoproteinemic as revealed by their hypercholesterolemia (Tables 1 and 2) shown by the data in **Table 3.** These data are only indicative, however, since assay conditions are rarely comparable and occasionally imprecise. In these fish, HDL dominate the lipoprotein profile, reaching considerable values in the Teleostei, more than 2000 mg/dl of plasma. As the result of the absence, still controversial, of an albumin-type plasma fraction in the Agnatha and Elasmobranchii (see ref. 18 for review), or its quantitatively nonpredominant presence in the Teleostei (19–22), lipoproteinemia is associated with low protein levels, contrary to that in mammals. This results in a hyperapolipoproteinemia; the plasma concentration of apolipoproteins accounts for about 36% of plasma proteins in rainbow trout (23) and 30% in channel catfish (17), whereas this figure is lower than 10% in humans.

In females of oviparous species, lipoproteinemia depends on the concentration of circulating vitellogenin (Table 3), which is a very high density lipoprotein (VHDL d > 1.21 g/ml), synthesized by liver cells under the control of estrogens and specifically incorporated by growing egg cells to be cleaved into yolk proteins. It has been detected in the blood of numerous fish species during their normal reproduction cycle or in response to estrogen stimulation (see refs. 24 and 25 for reviews).

B. Physicochemical characterization of plasma lipoproteins

Fish lipoproteins can be isolated by selective precipitation. The use of precipitation with dextran sulfate and manganese chloride, developed for human lipoproteins (26), has been used to precipitate plasma VLDL and LDL in the rainbow trout (27). The isolation of a globulin fraction with ammonium sulfate has enabled these lipoproteins to be detected in carp (28). In this species, the different lipoprotein classes have also been separated and characterized by high performance liquid chromatography (HPLC) on gel permeation columns (29, 30).

Nevertheless, lipoproteins are usually isolated by sequential ultracentrifugation flotation. This method must take into account the basal density of the serum, which is identical to that of humans in the case of catfish (17) but different in the case of rainbow trout (31) and certain sharks (32). Using this method, chylomicrons, VLDL, LDL and HDL could be isolated from all the species studied. These particles can be observed in the electron microscope and mean diameter can be determined.

The lipoproteins thus isolated can be characterized by their electrophoretic migration on agarose gels. This migration is highly anodic in the Pacific sardine (33) and the trout (16); compared with the migration described for human lipoproteins, HDL and LDL have an α migration. It is LDL that migrate the most in rainbow trout (16). The presence of a large quantity of plasma lipoproteins with high electrophoretic mobility is characteristic of a large number of fish species (23, 28, 31–38).

1. Lipids and lipid-soluble substances

The percentage of total lipids in each class of lipoproteins is comparable to that observed for human lipoproteins (**Table 4**). Fish can nevertheless be grouped into three broad distinct classes: Agnatha, Chondrichthyes, and Osteichthyes, each with their own characteristics.

In the Agnatha, there is hyperlipoproteinemia and hypercholesterolemia; HDL are abundant and VLDL and LDL predominate (Tables 1 and 3). Lipoproteins

TABLE 1. Total cholesterol content and percentage of cholesterol esters in the blood plasma of fish (n = number of animals)

Species	Total Plasma Cholesterol Mean ± SEM		% Cholesteryl Esters	Ref.
AGNATHA	mg/dl			
Mordacia mordax	613	(n= 4)	84	40
GNATHOSTOMES				
CHONDRICHTHYES				
ELASMOBRANCHII				
- **Rajidae**				
Raja lintea	86 ± 32	(n= 3)	80	229
Raja radiata	161 ± 57	(n=11)	72	229
- **Dasyatidae**				
Potamotrygon motoro	108 ± 29	(n= 4)		242
- **Carcharhinidae**				
Aprionodon isodon	111		43	243
Carcharchinus limbatus	154		45	243
- **Sphyrnidae**				
Sphyrna tiburo	254	(n= 2)	43	243
- **Heterodontidae**				
Heterodontus phillipi	72			35
- **Squalidae**				
Etmopterus spinax	214 ± 107	(n=11)	79	229
Squalus acanthias	179	(n= 2)		15
HOLOCEPHALI				
Chimaera monstrosa	474 ± 142	(n=14)	68	229
Hydrolagus colliei	97			244
OSTEICHTHYES				
GINGLYMODI				
- **Lepisosteidae**				
Lepisosteus productus	143	(n= 2)	75	243
Lepisosteus spatula	157		55	243
Lepisosteus osseus	154	(n= 2)	58	243
TELEOSTEI				
ELOPOMORPHA				
- **Elopidae**				
Elops saurus	1440		60	243
- **Anguillidae**				
Anguilla rostrata	816 ± 54	(n=10)		245
Anguilla anguilla	418 ± 22	(n= 8)	65	246
CLUPEOMORPHA				
- **Clupeidae**				
Brevoortia patronus	344	(n=30)	84	243
Dorosoma petenense	205	(n=25)	37	243
Dorosoma cepedianum	300	(n=20)		247
Clupea harengus	649 ± 62	(n=10)	69	229
OSTARIOPHYSI				
- **Cyprinidae**				
Cyprinus carpio	143 ± 28	(n=10)		29
Catla catla	151 ± 68	(n=59)	60	248
Carassius auratus	329 ± 10	(n=56)		249
- **Ariidae**				
Galeichthys felis	350	(n= 5)	81	243
Bagre marina	385	(n= 5)	92	243
- **Siluridae**				
Heteropneustes fossilis	588 ± 8	(n=12)		250
- **Claridae**				
Clarias batracus	643 ± 224	(n= 9)		251
PROTACANTHOPTERYGII				
- **Salmonidae**				
Salvelinus fontinalis	233 ± 19	(n= 6)		53
Salmo fario	381 ± 26	(n=10)	66	252
Salmo gairdneri	see Table 2			
Salmo salar	635 ± 56	(n= 7)		53
Oncorhynchus kisutch	670 ± 60	(n=17)		253
Oncorhynchus gorbuscha	267 ± 31	(n= 8)	54	230
Oncorhynchus tshawytsha	501	(n=21)	66	254
Oncorhynchus nerka	570 ± 19	(n=23)	54	255
- **Argentinidae**				
Argentina silus	772 ± 258	(n=11)	80	229
- **Esocidae**				
Esox lucius	121 ± 1	(n=10)		256
PARACANTHOPTERYGII	mg/dl			
- **Gadidae**				
Gaidropsarus cimbrius	248 ± 75	(n=10)	70	229
Merluccius merluccius	432 ± 69	(n=15)	70	229
Gadus poutassou	460 ± 118	(n=13)	68	229
Gadus aeglefinus	513 ± 57	(n=11)	69	229
Gadus virens	575 ± 218	(n=10)	68	229
Molva molva	660 ± 190	(n= 6)	68	229
Gadus merlangus	692 ± 108	(n= 8)	71	229
Gadus morhua	921 ± 416	(n=17)	66	229
- **Cyclopteridae**				
Cyclopteras lumpus	695 ± 67	(n= 3)	65	229
- **Lophiidae**				
Lophius piscatorius	190 ± 49	(n= 3)	88	229
- **Mugilidae**				
Mugil cephalus	560	(n=20)	71	243
ACANTHOPTERYGII				
- **Fundulidae**				
Fundulus heteroclitus	399 ± 18	(n= 5)		257
- **Serranidae**				
Hoccus americanus	422	(n=18)		247
Therapon bidyana	463 ± 55	(n=10)		35
Macquaria australasica	297 ± 19	(n= 4)		35
Oligorus macquariensis	464 ± 22	(n= 5)		35
Acanthistius serratus	238 ± 10	(n= 5)		35
- **Centrarchidae**				
Lepomis gibbosus	423	(n=11)		247
Micropterus salmoides	215	(n=36)		247
- **Sillaginidae**				
Silloago maculata	768 ± 128	(n= 3)		35
- **Pomatomidae**				
Pomatomus pedica	378 ± 15	(n= 6)		35
- **Lethrinidae**				
Pagrosomus auratus	336 ± 23	(n=10)		35
Girellidae				
Girella elevata	133 ± 13	(n= 3)		35
Girella tricuspidata	320 ± 31	(n= 4)		35
- **Carangidae**				
Caranx hippos	696		100	243
Seriola quinquer	152 ± 6			258
- **Sparidae**				
Lagadon rhomboides	904	(n=20)	99	243
Mylio australis	332 ± 38	(n= 5)		35
Archosargus probatocephalus	595	(n= 5)	70	243
- **Leiognathidae**				
Zeus australis	111 ± 9	(n= 6)		35
- **Coryphaenidae**				
Coryphaenoides rupestris	236 ± 75	(n=18)	69	229
- **Otolithidae**				
Cynoscion nebulosus	694	(n= 3)	59	243
Cynoscion arenarius	358	(n= 8)	47	243
- **Sciaenidae**				
Sciaenops ocellata	525	(n= 2)	81	243
Sciaena antarctica	404 ± 40	(n= 4)		35
Pogonias cromis	386		88	243
- **Triglidae**				
Trigla gurnardus	350 ± 69	(n= 3)	70	229
- **Scombridae**				
Scomberomorus maculatus	690		100	243
Scomber scombrus	358 ± 52	(n= 4)	62	229
Sarda chiliensis	383 ± 28	(n= 6)		35
Anabantidae				
Anabas testidineus	373	(n= 6)	34	241
- **Labridae**				
Achoerodus gouldii	87 ± 9	(n= 4)		35
Ophthalmolepis lineolatus	144 ± 27	(n= 6)		35
- **Cheilodactylidae**				
Nemadactylus douglashi	246 ± 23	(n= 5)		35
Ophiocephalidae				
Channa punctatus	370		40	259
- **Pleuronectidae**				
Glyptocephalus cynoglossus	418 ± 140	(n=11)	71	229
Pleuronectes platessa	658 ± 182	(n=12)	68	229

with β and pre-β migration after agarose electrophoresis have been identified in the lamprey *Petromyzon marinus* (39). Data obtained with *Mordacia mordax* (40) have shown that there is a paucity of triglycerides in all three classes of lamprey lipoproteins, while cholesterol is highly abundant in the light lipoproteins, in particular LDL, where it accounts for 70%, primarily in the esterified form. The composition of HDL is very special, suggestive of nascent hepatic HDL in mammals. Phospholipids are the major lipid; cholesteryl esters account for less than 6% of lipids, while triglycerides are practically absent. The fatty acid composition of the lipoproteins is comparable to that of

TABLE 2. Plasma cholesterol in rainbow trout (*Salmo gairdneri*)

Total Cholesterol		Comments		Ref.
mg/dl				
161–365	(n = 200)	juvenile	(100 g)	260
299	(%CE 73)		(200 g)	261
303	(%CE 65)	juvenile	(140–200 g)	13
280 ± 13	(n = 22)	male	(180–250 g)	262
298 ± 12	(n = 25)	female	(180–250 g)	262
289 ± 64	(n = 7)	male, female	(450–550 g)	53
235	(%CE 55)	male	(506–1038 g)	16
297 ± 97	(n = 6)	male, female	(700–1000 g)	31
500	(n = 3)	female	(700–1000 g)	31
524	(n = 13)	male immature	(200–400 g)	263
442	(n = 10)	female immature	(200–400 g)	263
432	(n = 6)	male mature	(1585 g)	263
361	(n = 7)	female mature	(1585 g)	263
362	(n = 13)	male migrating	(1360–4750 g)	263
225	(n = 25)	female migrating	(1360–4750 g)	263
712 ± 175	(n = 8)	male immature	(400–1230 g)	264
744 ± 182	(n = 6)	female immature	(630–1490 g)	264
546 ± 200	(n = 14)	male mature	(395–2600 g)	264
474 ± 172	(n = 16)	female mature	(508–1890 g)	264
522 (148–1000)	(n = 70)	different	(78–290 g)	265
604 (170–1322)	(n = 70)	population with	(112–412 g)	265
355 (96–734)	(n = 70)	different	(64–284 g)	265
455 (141–827)	(n = 70)	diet	(60–406 g)	265
416 ± 31	(n = 7)	fed	(200–300 g)	149
315 ± 22	(n = 7)	starved for 8 weeks		149

%CE, % cholesteryl esters; standard values in mammals, 100–250 mg/dl.

the Chondrichthyes and Osteichthyes: saturated fatty acids are primarily palmitic and stearic and the unsaturated fatty acids belong to the n-6 and n-3 families. The essential characteristic is the abundance of polyunsaturated fatty acids, particularly 20:4 (n-6) (in phospholipids), 20:5 (n-3) and 22:6 (n-3) (in triglycerides and cholesteryl esters), an abundance encountered in the muscle and liver (40).

At the onset of spawning migration, the female river lamprey has a significantly higher concentration of nonultrafilterable calcium than the male (41). This higher concentration is due to the presence of a phospholipoprotein type yolk precursor which binds calcium, in transit between the liver and the ovaries. This vitellogenin has been demonstrated immunologically in the blood of sexually mature females (42, 43) and induced in adult male or immature animals by 17β-estradiol (44).

In the Chondrichthyes, data obtained in different species are very coherent. In comparison to standards applied to humans, for example, the group is characterized by normal lipoprotein and cholesterol levels combined with a very low concentration of HDL, rarely exceeding 10% of total lipoproteins. In most elasmobranch species studied, chylomicrons could not be detected, but they have been reported in low quantities in the serum of *Centrophorus squamosus* (45) and account for 25% of plasma lipids in *Squalus acanthias* (15). They are probably present in the plasma during the postprandial phase in all fish- and plankton-eating species.

Each class of lipoprotein (VLDL, LDL, HDL) in this group contains the usual components of corresponding mammalian lipoproteins, but is distinguished from the latter by the presence of notable quantities of hydrocarbons and monoalkyldiacylglycerols (**Table 5**). These have been observed in all species studied (30, 45, 46), except *Scyliorhinus canicula* (45). For this species, it is believed that monoalkyldiacylglycerols are undoubtedly present, but are assayed with triglycerides, since the methodology used did not permit their separation. Electron microscopic examination in *Centrophorus squamosus* has revealed spherical VLDL and LDL. The VLDL fraction is very heterogeneous; particles in the LDL fraction are much more homogeneous with a mean diameter of 23.8 nm, in the range of 17.5 to 30 nm. These particles are thus morphologically comparable to human LDL, and their proportions of proteins, phospholipids, and free cholesterol are the same (Table 5). If we assume a direct relationship between the sum of these hydrophilic surface constituents and particle diameter, we may assume that hydrocarbons and glycerol

TABLE 3. The concentration of plasma lipoproteins in fish (mg lipoprotein/dl plasma)

Species	VLDL	LDL	HDL	Comments	Ref.	Vitellogenin		Ref.
AGNATHA								
Mordacia mordax (lamprey)	226	664	507	spawning migration	40 [a]			
GNATHOSTOMES								
CHONDRICHTHYES								
Centrophorus squamosus (shark)	415	230	40		45			
Centrophorus granulosus (shark)	265	134	23		46			
Centroscymnus coelolepis (shark)	196	145	38		46			
Scyliorhinus canicula (dogfish)	28	154	23		266	(♀)	40	51
Squalus acanthias (spiny dogfish)	307	120	33	2.5-3.2kg	15			
OSTEICHTHYES								
ACTINOPTERYGII								
PALEOPTERYGII								
CHONDROSTEI								
Acipenser stellatus (sturgeon)	348	23	204		46			
Acipenser guldenstadtii (sturgeon)	496	164	672		46			
Huso huso (sturgeon)	726	510	649		46			
NEOPTERYGII								
TELEOSTEI								
- Elopomorpha								
Conger vulgaris (conger eel)	456	225	n.d.		266			
- Clupeomorpha								
Sardinops caerulea (pacific sardine)	115	145	1120		33 [a]			
- Ostariophysi								
Cyprinus carpio (carp)	-	56	981	300-500g	29 [b]			
Ictalurus punctatus (channel catfish)	507	338	1764	♂ mature, 1-2kg	17 [a]			
- Protacanthopterygii								
Salmo gairdneri (rainbow trout)	201	392	2216	1 ♀ 1 ♂ 1-1.6kg	52 [a]			
	212	193	1062					
	30	186	1500	0.7-1kg	31 [a]			
	586	1156	518	pool 15 ♂, 0.5-1kg	16 [a]			
	335	1189	331	pool 50, immature 100-120g, 1 year old				
	171	879	1371	pool 50 ♂, spermation 100-120g, 1 year old	212 [a]			
	47	441	1013	pool 50 ♂, spermation 1-1.2kg, 3 years old				
	650	700	1750	pool 8 ♀, 1-1.2kg		(♀)	3170	69 [a]
	100	550	1300	pool 8 ♀, ovulation	69 [a]	(♀)	6580	
						(♀)	10-1290	235
						(♂)	<2	141
						(♀)	1-5000	237
Salmo salar (atlantic salmon)						(♀)	1- 600	268
						(♂)	47	133
						(♀)	12-2160	
Oncorhynchus nerka (sockeye salmon)	167	246	238	juvenile	111 [a]			
Oncorhynchus gorbuscha (pink salmon)	-	-	3300	spawning	37	(♂)	<1	269
						(♀)	1-900	
Oncorhynchus keta (chum salmon)						(♀)	100-2260	238
Esox lucius (pike)						(♀)	130-4820	140
- Paracanthopterygii								
Gadus morhua (cod)						(♀)	3200	270
CROSSOPTERYGII								
Latimeria chalumnae (coelacanth)	1105	194	127		59			
MAN (urban)	132	374	230	fasting males	267			

Distributions were normally determined by analytical ultracentrifugation of pools of serum or plasma (45), by lipoprotein recoveries after centrifugal flotation (a), or by lipoprotein separation after high performance liquid chromatography (b). Values in *Sardinops caerulea* and in *Cyprinus carpio* were calculated assuming protein contents of 10% in VLDL, 20% in LDL, and 50% in HDL. Levels of vitellogenin were generally determined by immunological methods (radioimmunoassay, immunodiffusion) and are expressed in terms of protein content only, except for *Salmo gairdneri* in reference 69; n.d., not determined.

ethers are in the core of the particle along with other neutral lipids (cholesteryl esters and triglycerides) and compensate for the low level of cholesteryl esters. The structure of the lipoprotein envelope is thus the same as in mammals. The low density of squalene (0.86 g/ml), the essential constituent of the hydrocarbons, and the alkyldiacylglycerols (0.91 g/ml), their inefficient utilization, and also their storage in the liver have led some authors

TABLE 4. Physicochemical properties of human and trout plasma lipoprotein families

	Electrophoretic mobility		Particle size (nm)		Molecular weight $\times 10^{-6}$		Density range (g/ml)		Lipid (% wt.)		Protein (% wt.)		Major apolipoprotein		Minor apolipoprotein	
	Man	Trout	Man	Trout	Man	Trout	Man	Trout	Man	Trout	Man	Trout	Man	Trout[c]	Man	Trout[c]
Chylomicrons	origin	origin	75–1200	80–800	400		< 0.930	< 1.015	98	95.5	2	4.5	A-I,A-IV,B, CI,CIII,E	25(A-I), B_{240}, B_{260}, 76, 13(A-II), 9-11(C)	A-II,CII	
VLDL	pre-β	pre-β	30–80	20–50 (30)	10–80		0.930–1.006	< 1.015	92	87.2	8	12.8	B,E, CI, CII, CIII		A-I,A-II, A-IV	
IDL	slow-pre-β	α	25–35	12–21 (18)	5–10		1.006–1.019	1.015–1.040	82	70.5	18	29.5				
LDL	β	α	18–25		2.3		1.019–1.063	1.040–1.085	79		21		B	$B_{240,76}$	CI,CII CIII,E	25(A-I)
HDL_2	α		9–12		0.360		1.063–1.125		55		45		A-I,A-II,	25(A-I), 13(A-II)	A-IV,E, CI,CII, CIII,D, F	55(A-IV), 40.5, 9-11(C)
HDL		α		6–11 (8)		0.180[b]		1.085–1.210		55.3		44.7				
HDL_3	α		5–9		0.175		1.125–1.210		45		55					
Vitellogenin					0.440 (native dimer)		> 1.210		18		82		175 (monomer)			

Recapitulation from references 10, 125, 156, 271, 272 from human lipoproteins and from references 16, 21, 23, 31, 52, 69, 70, 212, 214 for rainbow trout (*Salmo gairdneri*) lipoproteins.
[a]Major apolipoprotein" refers to proteins comprising 5% or more of the total protein in plasma lipoproteins.
[b]25: Apolipoprotein of molecular weight 25,000, etc.
[c]Value for HDL in pink salmon (*Oncorhynchus gorbuscha*) (37).

(see ref. 47 for review) to suggest that these two constituents play a basic role in the hydrostatic equilibrium of elasmobranch fish with no swim bladder.

The distribution of fatty acids is comparable to that described above for the lampreys. The basic characteristic is the abundance of polyunsaturated fatty acids, in particular 20:5 (n-3) and 22:6 (n-3), in the cholesteryl esters of the three classes of lipoproteins (45). Although wax esters are detected in only trace quantities in *Centrophorus squamosus* (46), older work (32) had estimated them at between 9 and 32% of neutral lipids in the serum of four shark species. The explanation for this difference is undoubtedly that in the latter case (32) thin-layer chromatography was used with n-hexane–diethyl ether 19:1 (v/v) as developing solvent. In this system cholesteryl esters whose fatty acid is 22:6 (n-3) separate from those with shorter and less unsaturated fatty acid chain and migrate with wax esters.

In the oviparous species *Scyliorhinus canicula,* estradiol treatment increases the plasma calcium concentration (48) and also that of a plasma lipophosphoprotein. The antigenic similarity of this protein with vitellin granules in oocytes has led to it being considered as a vitellogenin (49–51). Its concentration in females remains low (0.4 mg/ml) throughout the annual reproduction cycle and it contains 18% lipids, half of which are phospholipids (51).

In Osteichthyes, with the exception of the coelacanth, HDL tend to dominate the lipoprotein profile in both Paleopterygii and Neopterygii; data exist only in Teleostei (Table 3). As an example, the chemical composition of the different classes of plasma lipoproteins of the rainbow trout is given in Table 5. The light lipoproteins (chylomicrons, VLDL, and LDL) have more surface constituents than their human counterparts and thus they are smaller (Table 4). Trout serum is denser than human serum and the particles are smaller than in humans; thus trout LDL are distributed up to d 1.085 g/ml (23, 31, 52). With regard to HDL, the proportions of core and surface constituents are the same as in humans and their size is thus similar to that of human HDL_3. The ratio of cholesteryl esters to free cholesterol is about 2 in trout lipoproteins, while it increases from 2 to 5 from VLDL to HDL in man. The hydrophobic core of trout LDL and HDL is thus comparatively enriched in triglycerides and depleted in cholesteryl esters. In salmonids, most plasma cholesterol is transported by HDL as a result of their abundance (53, 54) and cholesterol levels are high (Tables 1 and 2); in man, cholesterol is transported mainly in LDL (10) (cholesterol VLDL + LDL/cholesterol HDL = 0.7 in trout and 3 in man).

The most abundant phospholipid in salmonid lipoproteins (37, 55) and in the Pacific sardine (33) is phosphatidylcholine, as in humans. The same is true for sturgeon LDL (45).

TABLE 5. Chemical composition of fish and human plasma lipoproteins (% by weight)

Fraction	Chylomicrons		VLDL				LDL				HDL				Vitellogenin
	Trout	Human	Lamprey	Shark	Trout	Human	Lamprey	Shark	Trout	Human	Lamprey	Shark	Trout	Human	Trout
Core lipids															
Triglyceride	84	80–95	36	23	52	45–65	2	8.7	22.3	4–8	0.4	3.4	11.1	2–7	4
Cholesteryl ester	2.2	2–4	31	21.4	11	16–22	60	25.9	14.9	45–50	5.6	13.9	9.1	15–20	2[a]
Monoalkyldiacyl glycerol				8.2				10.9				8			
Hydrocarbon				18.8				5.9				6.9			
Surface components															
Free cholesterol	1	1–3	5	6.7	5.7	4–8	10	8.4	6.3	6–8	4.2	3.8	3.4	3–5	
Phospholipid	8.3	3–6	20	15.3	18.5	15–20	11	20.5	27	18–24	46.8	14.5	31.7	26–32	11
Protein	4.5	1–2	8	3.1	12.8	6–10	17	17.6	29.5	18–22	43	47.7	44.7	45–55	82

Reference(s): *Mordacia mordax*, lamprey (40); *Centrophorus squamosus*, shark (45); *Salmo gairdneri*, rainbow trout (212) chylomicrons. Averaged data from (16, 31, 52) for rainbow trout VLDL, LDL, and HDL; from (70) for rainbow trout vitellogenin; from (272) for human lipoproteins.
[a]Total cholesterol.

Wax esters are probably transported by plasma lipoproteins, even though they could not be detected in the Pacific sardine (33) or the rainbow trout (31). Their presence in the lipoproteins of this species has been mentioned (27) and small quantities (0.34 mg/ml) have been detected in the serum (56). The plasma carries wax esters in the carp (30, 57). However, the report that 14.5 to 16.1% of lipids of the different lipoprotein classes are wax esters (58) should be taken with reservations, since their separation from sterol esters is not obvious.

The fatty acid composition of the esters of the different classes of lipoproteins again shows the high proportion of very polyunsaturated fatty acids of the n-6 (20:4) and n-3 (20:5 and 22:6) families. This is thus a basic characteristic of all fish, except for the coelacanth (59). Fish are poikilothermic animals and the high degree of fatty acid unsaturation in membrane phospholipids enables cell membrane fluidity to be maintained even at low temperature (60). Two essential processes have been demonstrated in rainbow trout: elongation–desaturation of fatty acids accompanying acclimatization to cold (see refs. 61 and 62 for reviews) and the cycle of temperature-dependent deacylation/reacylation of phospholipids (63). In the trout, the degree of unsaturation of all lipoproteins is high, since more than 60% of fatty acids are unsaturated (see ref. 4 for review). These lipids are very rich in 18:1 (n-9) and in longer-chain polyunsaturated fatty acids of the n-3 series, in particular 22:6 (n-3). This fatty acid predominates in the cholesteryl esters and phospholipids of the three lipoprotein classes and represents 29% (by weight) of the HDL fatty acids, 21.8% of LDL acids, and 16.8% of VLDL acids (52). The fatty acids in this series are essential and indispensable for the survival and normal growth of the fish (see refs. 5, 6 and 64 for reviews), while land vertebrates require especially the n-6 series. The efficiency of 18:3 (n-3) as essential fatty acid is the same as that of 20:5 (n-3) or 22:6 (n-3) in fresh water fish as the rainbow trout, the ayu, and the eel. On the contrary, it is not very effective in salt water fish such as the turbot or chad. This is related to the fact that the true essential fatty acid in fish is 22:6 (n-3) and its synthesis from 18:3 (n-3) is high in fresh water fish and low in the turbot and chad (see ref. 62 for review). This fatty acid is transformed by elongation and desaturation, essentially in the liver (65–67). The role of lipoproteins, in particular phospholipids and cholesteryl esters, is thus fundamental in the transport of 22:6 (n-3), a role comparable to that of mammalian lipoproteins for the transport of 20:4 (n-6). It should be remembered that for fatty acids with the same carbon chain length, the melting point of the n-3 series is lower than that of the n-6 series.

Vitellogenin has been demonstrated in the plasma of numerous teleost species. It is a VHDL whose lipid content has been reported as 21.5% (68, 69) and 18% (70) in rainbow trout (Table 5), 19% (70) in sea trout and 21% and 20% in goldfish (71, 72). In all cases, phospholipids predominate and represent, by weight, about two-thirds of these lipids (69–71). In a manner comparable to other classes of lipoproteins, trout vitellogenin is very rich in polyunsaturated fatty acids (69, 73), in particular 22:6 (n-3) which accounts for about 20% of the total fatty acids (73).

Vitellogenin is a lipophosphoglycoprotein complex that binds calcium. In trout, for example, it contains 0.5% calcium (74) and 0.6% phosphoprotein phosphorus (68, 70), combined with a very serine-rich section of the protein, phosvitin (75–80). This highly phosphorylated part has been used for the in vivo labeling of vitellogenin after injection of [^{32}P]orthophosphate in certain fish (70, 72, 81–83). As a result of these quantitative variations, vitellogenin has very often been assayed indirectly in fish by measuring the plasma levels of phosphoprotein phosphorus (84–89), alkali-labile phosphoprotein phosphorus (68, 71, 88–98), or calcium (71, 84–87, 89, 99–101) both in

females during vitellogenesis and in animals receiving estrogen. Fish vitellogenin can also bind iron (102, 103) and magnesium (101).

Plasma lipoproteins transport lipid-soluble substances in the plasma. In the course of sexual maturation of salmonids, carotenoids, particularly astaxanthine, are transferred by the plasma from the muscles where they are stored to the skin and gonads (104, 105). Astaxanthine is transported by HDL in the plasma of the chum salmon *Oncorhynchus keta* (106–108) and also by vitellogenin (109) during spawning migration. In the trout, vitamin E (α-tocopherol) circulates in the plasma preferentially combined with LDL (110). The protective effect of tocopherol against lipid peroxidation in fish is most probably essential, because the polyunsaturated fatty acids are particularly sensitive to autooxidation. Other lipid-soluble substances, such as inorganic mercury ($HgCl_2$) or methylmercury (CH_3HgCl) may combine with plasma lipoproteins. In the sockeye and coho salmon, *Oncorhynchus nerka* and *kisutch,* these substances are incorporated in lipoproteins (111) and their interaction with salts of heavy metals (Hg, Cd) changes the surface structure of the lipoprotein (112).

2. Apolipoproteins

Both the nature and the distribution of apolipoproteins in different classes in fish resemble that of mammals (2, 113, 114). Thus, an apoB-like protein is the major species in VLDL and LDL and apoA-like proteins predominate in HDL (Table 4).

In an Agnatha such as the lamprey *Petromyzon marinus,* the HDL contain two apolipoproteins present in high concentrations. Their sequence has been determined from liver cDNA library (115). They were named LAL_2 and LAL_1. The two mature apolipoproteins are composed of 168 and 76 residues, respectively, and have theoretical molecular weights of 18,123 and 8,793. In both cases, the sequences are largely helix-permissive and contain a region coding a peptide containing 23 residues in LAL_2 and 21 residues plus a putative 8-residue propeptide in LAL_1. Both apolipoproteins are also characterized by the lack of cystine. Sequence alignment between human and lamprey apolipoproteins has revealed that lamprey LAL_1 has a repeat pattern similar to that in human apoA-II and C-III (116). The length of mature peptide and the presence of prosegment in LAL_1 lead to the conclusion that LAL_1 could be the counterpart of mammalian apoA-II (116).

In the Gnathostomes, the often fragmentary data concerning the protein moiety of plasma lipoproteins are currently limited to the shark *Centrophorus squamosus* (45, 46, 117, 118) for the Chondrichthyes and to eight species of teleost fish for the Osteichthyes (17, 21, 23, 28, 31, 37, 52, 108, 119–123). Preliminary data have been obtained for the apolipoproteins of the carp *Cyprinus carpio* (28) and the eel *Anguilla japonica* (120), where a 78,000 molecular weight plasma apolipoprotein is combined with triglycerides, fatty acids, cholesterol, and biliverdin.

The presence of an apolipoprotein equivalent to human apoB in the low density lipoproteins of fish has been suggested by the insolubility of a fraction of their protein moiety after delipidation and its solubilization in amphipathic detergents such as sodium dodecyl sulfate (SDS). Using SDS-polyacrylamide gel electrophoresis (PAGE), a high molecular weight component (> 250,000) was identified in the VLDL and LDL of the shark *Centrophorus squamosus* (45), the trout *Salmo gairdneri* (31, 52), the salmon *Oncorhynchus tshawytscha* (121), and the catfish *Ictalurus punctatus* (17, 123). In trout, the presence in VLDL and IDL of two apoB-like proteins of M_r 260,000 and 240,000 has been demonstrated, while only an apoB-like protein of M_r 240,000 is present in the LDL (21, 23, **Fig. 2**). Fractionation by gel filtration chromatography followed by amino acid analysis of the high molecular weight apolipoproteins of shark LDL (46) and the VLDL and LDL of trout (31) indicated that there was considerable similarity to the profile of human apoB (see ref. 2 for review). After SDS-glycerol-PAGE of density gradient ultracentrifugation fractions and staining with Coomassie Blue, the integration of peak surfaces of densitometer scans showed that the two apoB-like proteins of trout accounted for 30–65% of total lipoproteins, from VLDL to LDL (122). This is in agreement with prior determinations by gel filtration chromatography (31). A weak immunological cross-reaction has been observed between the LDL of the shark *Centrophorus squamosus* and an antiserum against human LDL and apoB (117, 118), as well as between human VLDL and LDL and an antiserum against trout VLDL and LDL (31). The presence of common antigenic determinants in human apoB and in trout apoB-like protein of M_r 240,000 and 260,000 has been shown by immunoblotting (**Fig. 3**). Immunodetection, however, is possible only with high concentrations of antigens and specific anti-human apoB antibody, indicating that only a certain number of epitopes have been retained. Apolipoprotein B is a fundamental lipid-transporting protein in which at least one part of its structure has remained unchanged in the course of vertebrate evolution (117, 124).

Salmonids and trout, in particular, are the best known apolipoprotein models among fish. As seen in Fig. 2, trout VLDL and IDL contain, in addition to two apoB-like proteins, a third apolipoprotein of M_r 76,000, which is only present in LDL with the apoB-like protein of M_r 240,000. Type A apolipoproteins are also present in VLDL and IDL in addition to a group of apolipoproteins of M_r 9,000–11,000 (apoC-like). The HDL contain four apolipoproteins, two major types of M_r 25,000 (apoA-I-like (65%)) and M_r 13,000 (apoA-II-like (33%)) and two

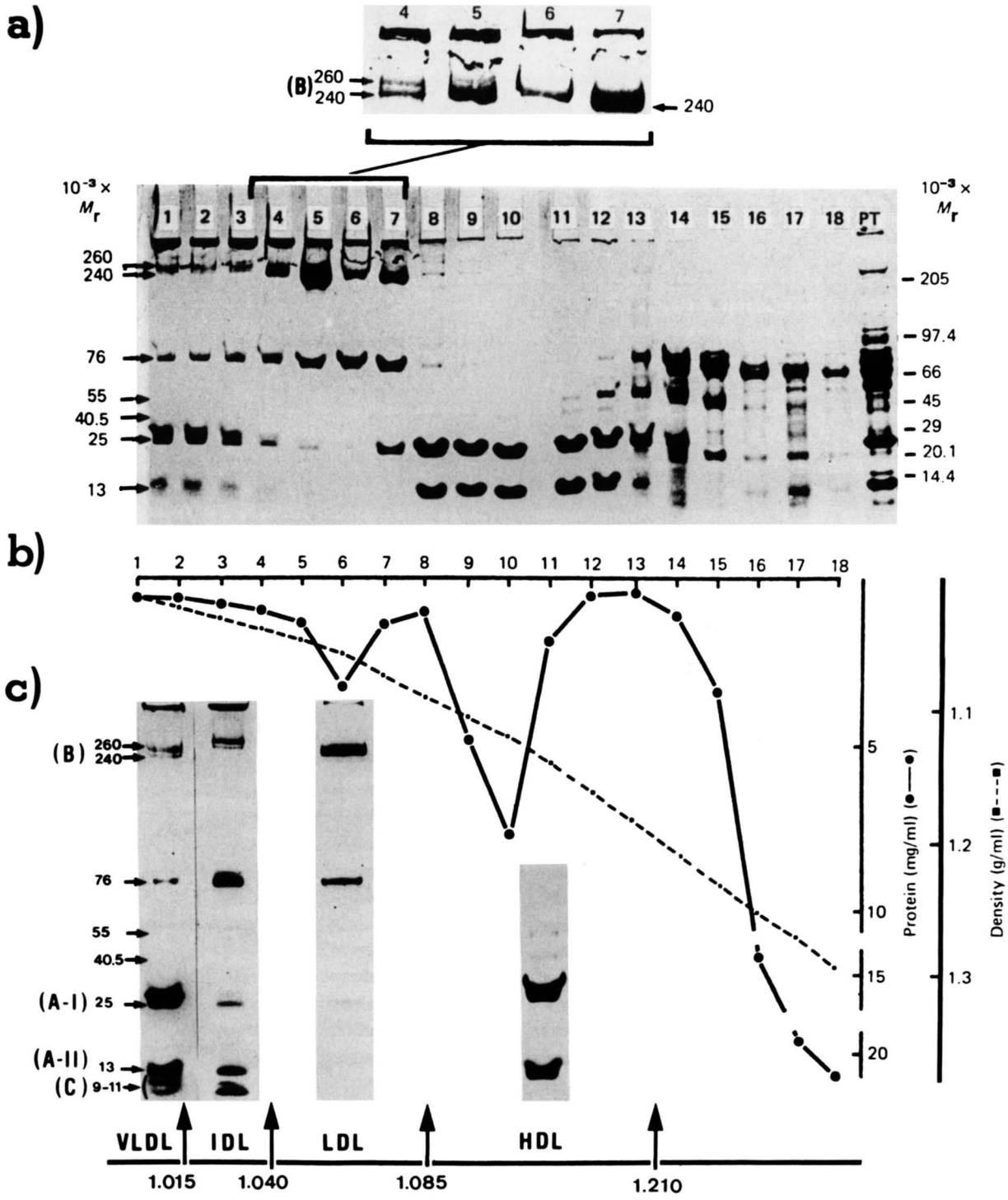

Fig. 2. Plasma apolipoprotein distribution in the trout as a function of lipoprotein density (see refs 21 and 23 for details). a) Electrophoretic patterns in SDS-glycerol-polyacrylamide gel slabs (linear gradient 3.5–15% polyacrylamide and 8–12% glycerol) of trout apolipoproteins from density gradient ultracentrifugation subfractions 1–18 and total plasma proteins (PT). The M_r values ($\times 10^{-3}$) of the apolipoproteins are on the left and the standards on the right; staining with Coomassie Blue R-250. The present work involved fractionating the plasma of female trout in previtellogenesis. Vitellogenin (monomer = M_r 175,000) may be considered as a VHDL (d > 1.21 g/ml) and is thus not observable. b) (■ — ■), Density profile determined from control density gradient subfractions containing only NaBr solutions; (● — ●), lipoprotein profile as evaluated by protein content in each fraction of the density gradient. The abscissa is the number of successive fractions from the top of the tube. c) Electrophoretic patterns in SDS-glycerol-polyacrylamide gel of the apolipoproteins of the principal classes of plasma lipoproteins isolated by sequential ultracentrifugation flotation. Staining was with Coomassie Blue R-250. Left to right: apoVLDL (d < 1.015 g/ml); apoIDL (1.015 < d < 1.040 g/ml); apoLDL (1.040 < d < 1.085 g/ml); apoHDL (1.085 < d < 1.21 g/ml). The M_r values ($M_r \times 10^{-3}$) of the apolipoproteins are on the left.

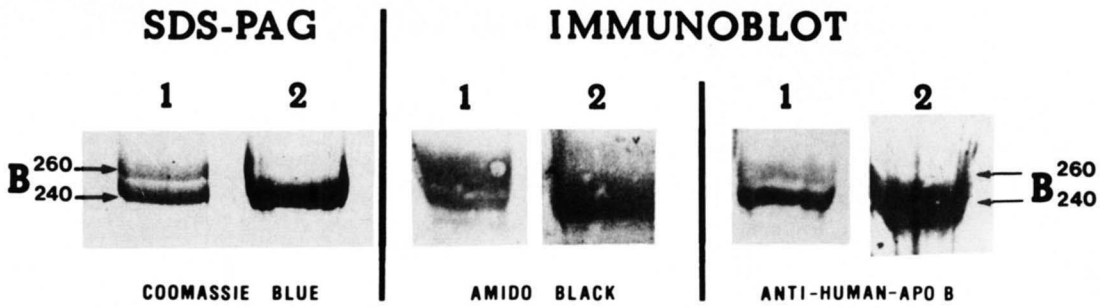

Fig. 3. Immunoblotting showing the cross-reaction between trout apoB and human apoB (unpublished data). Common antigenic determinants are identified by Western immunoelectroblotting for apolipoproteins of VLDL (lane 1) and of LDL (lane 2) of trout using monospecific anti-human apolipoprotein B antiserum (Boehringer-Mannheim Cat. no. 726494). Trout lipoproteins were isolated by density gradient ultracentrifugation (fraction 1 for VLDL and fraction 6 for LDL (23)) and their apolipoproteins were separated by SDS-glycerol-PAGE in a linear gradient of 3.5–15% polyacrylamide/8–12% glycerol as described (21). Electroblotting on polyvinylidene difluoride membranes (PVDF-Millipore) was in 25 mM Tris/192 mM glycine buffer, pH 8.3, and 20% v/v methanol for 1.5 hr at 1 mA/cm^2. The two trout apoB-like species of M_r 260,000 and 240,000 are identified on the left after staining the gel with Coomassie Blue. The complete transfer of the proteins was confirmed by the absence of residual apolipoproteins in the gel after staining and by their presence on the membrane (middle), seen after staining with Amido Black. The cross-reaction is shown on the membrane (right side) by the double antibody technique as described (21). Briefly, after incubating the membrane with anti-human apoB serum raised in sheep, a rabbit anti-sheep IgG serum coupled with horseradish peroxidase was used. Detection was with 4-chloro-1-naphthol as chromogen.

minor types (M_r 55,000 (0.8%) and 40,500 (1.2%)). As a result of the predominance of HDL in adult trout, the apoA-I-like protein is one of the most abundant plasma proteins. The concentration of this apolipoprotein in plasma of this species is about 12 mg/ml (23); the corresponding value in humans is 1.3 mg/ml (125). The accumulation of apolipoproteins of M_r 55,000, 76,000, and type A apolipoproteins in the d 1.21 g/ml infranate in trout suggests the presence of VHDL and/or free apolipoproteins, in particular an apolipoprotein of M_r 55,000, whose distribution is similar to that of human apoA-IV (21, 23). This agrees with published data on fish, particularly for the two type A apolipoproteins of trout HDL (31, 52). These two apolipoproteins have also been identified in the HDL of other fish species, e.g., cod *Gadus morhua* (119), salmon (*Oncorhynchus gorbuscha* (37), *tschawytscha* (121), and *keta* (108) (M_r 24,000 and 12,000 in SDS-PAGE), and catfish *Ictalurus punctatus* (17,123) (M_r 25,000 in SDS-PAGE for the apoA-I-like protein). Size determination by electrophoresis or chromatography thus indicates that the molecular weights of these apolipoproteins in fish are similar to mammalian apoA-I and A-II (114). Human apoA-I and the corresponding trout or pink salmon apolipoprotein have a similar amino acid composition (37, 52). In fish, isoleucine is absent, aspartic acid and leucine levels are lower, and alanine is higher. In the salmon, different isoforms apparently exist (37, 121) similar to human apoA-I (see ref. 126 for review). Electrophoretic migration is unchanged before or after the reduction of possible disulfide bridges in the trout apoA-II-like protein (21, 31) and cystine is absent in the salmon apolipoprotein (37). This indicates a monomeric form as in most mammals, since the presence of dimeric apoA-II is limited to some species such as man and chimpanzees (114, 127). The electrophoretic behavior of low molecular weight trout and catfish apolipoproteins soluble in tetramethylurea, particularly those in VLDL, is not strictly comparable to that observed for human lipoproteins (17, 31). Nevertheless, the presence of the equivalent of human apoC-II is strongly suggested by the activation of trout lipoprotein lipase by these lipoproteins (see below).

In the course of vitellogenesis, vitellogenin is cleaved in the ovary into egg yolk proteins, lipovitellin and phosvitin, which accumulate in growing oocytes to be used for future embryonic development (see refs. 128, 129 for reviews and 21 for trout yolk proteins). The study of the apolipoprotein profile throughout the annual reproduction cycle in trout has shown the presence of yolk proteins in the plasma for the first time in an oviparous species. Yolk resorption by females after massive intraovarian follicular atresias, in particular after ovulation, results in the appearance of egg yolk proteins intimately combined with HDL in the plasma, which changes the apolipoprotein profile of this lipoprotein class (21, **Fig. 4**). Two egg yolk proteins combined with HDL have been identified. Lipovitellin (M_r 120,000) is composed of two subunits in 1:1 molar ratio (LV_1 with M_r 92,000 and LV_2 with M_r 20,000) and is present as a dimer with another yolk protein (M_r 10,000) (Fig. 4).

In teleost fish, plasma vitellogenin generally circulates as a dimer. Its molecular weight has been estimated in different species by gel filtration or polyacrylamide gel electrophoresis: in rainbow trout 600,000 (130) and 440,000 (68, 70, 131) by gel filtration, 535,000 (132) and 470,000 (68) by electrophoresis; 495,000 and 520,000 in the Atlantic salmon (133), 550,000 in flounder (90), and 350,000 in the eel (103) by gel filtration; 380,000 (72) and 326,000 (71) in goldfish, 640,000 in the stickleback (134) and 420,000 in the medaka (135) by electrophoresis. In some of these fish, the delipidated vitellogenin monomer

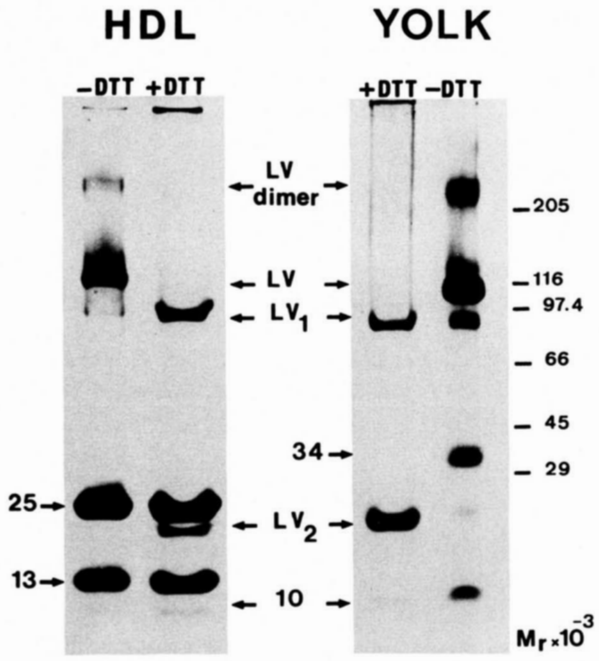

Fig. 4. Association of egg yolk proteins with plasma HDL after ovulation and in the course of follicular atresia in the trout. The HDL apolipoproteins of a female trout during follicular atresia were compared to total vitellin proteins by SDS-glycerol-PAGE in a linear gradient of 3.5–15% polyacrylamide and 8–12% glycerol; staining was with Coomassie Blue. The samples were treated with SDS alone (−DTT) or with SDS + dithiothreitol (+DTT) to reduce disulfide bridges. The M_r values of the standards are shown on the right and the identity of the proteins or their subunits is shown on the left and in the center. LV, lipovitellin; LV_1, lipovitellin 1; LV_2, lipovitellin 2; 34, β-component dimer, M_r 34,000; 10, M_r 10,000 protein; 25, apoA-I-like; 13, apoA-II-like; for more details see ref. 21. Reproduced with permission from the *Journal of Biological Chemsitry.*

was identified by SDS-PAGE. A single polypeptide appears to be present in trout (M_r estimated by different authors at 220,000 (130), 200,000 (136), 175,000 (21), and 170,000 (137)); in catfish (M_r 145,000 (138)); in killifish (M_r 200,000 (82)), in the stickleback (M_r 116,000 (139)); or the medaka (M_r 200,000 (135)). The presence in goldfish of a heterogeneous monomer with at least three polypeptides with M_r between 147,000 and 140,000 has been reported (72). In addition, in the eel *Anguilla japonica,* vitellogenin does not circulate in the plasma as a dimer, but is apparently constituted of four identical subunits of M_r 85,000 (103). Amino acid analysis indicates that there are considerable similarities between the vitellogenins of different fish species such as trout (68,130), goldfish (72), eel (103), or medaka (135). This results in the presence of common antigenic determinants in the vitellogenins of different fish species, revealed by cross-reactions using specific antibodies (139–141). This cross-reaction may be weak, however, even in very closely related species (68,133).

C. Plasma enzyme activities related to lipoprotein metabolism

The presence of a lipoprotein lipase (LPL) in different tissues of fish is related to the capacity of these tissues to hydrolyze triglycerides of circulating lipoproteins to their constituent fatty acids and glycerol moieties before their uptake by extrahepatic cells and tissues, e.g., muscle or adipose tissue. The intravenous injection of heparin in tilapia (142) and trout (143,144) leads to the appearance of lipase activity in the plasma. This lipoprotein lipase was purified from trout (144): its properties and molecular weight are similar to those of the mammalian enzyme (M_r 63,000 on SDS-PAGE and inhibition by 0.6 M NaCl and protamine sulfate). The enzyme is strongly activated by trout VLDL (144) and to a lesser extent by HDL (144, 145). Postheparin plasma of tilapia can also activate rat lipoprotein lipase (142). This suggests the presence of the equivalent of mammalian apoC-II in fish lipoproteins, the activating cofactor of lipoprotein lipase. Apolipoproteins with electrophoretic migration similar to human apoC have been identified in trout (21, 23, 31, 52, 145). LPL is attached to the surface of the vascular endothelium and is present in trout in numerous tissues: red and white muscle, heart, brain, liver, adipose tissue, and ovaries (143, 146–148) and in cod liver (148). In addition, a salt-resistant lipase activity, the counterpart of mammalian hepatic triglyceride lipase, is present in the livers of trout and cod (148) and also in extrahepatic tissues of trout: red and white muscle, heart, brain, adipose tissue, and ovaries (143, 146, 147, 149).

A lecithin:cholesterol acyltransferase activity (LCAT) has been shown in the plasma of the char *Salmo alpinus* (150), carp *Cyprinus carpio* (151), and trout *Salmo gairdneri* (152). This enzyme catalyzes the esterification of cholesterol in the plasma and uses apoA-I as cofactor. Contrary to the situation in humans, the trout or char enzyme is not reversibly inhibited by sulfhydryl blockers (153). Even though the presence of these two enzyme systems, LPL and LCAT, has been demonstrated in the plasma of some fish species, there as yet exist no data on the functional characterization of the apolipoproteins of these fish.

Similar to the activity of LCAT, which catalyzes the transfer of fatty acids in lecithin to cholesterol (154), a lecithin:alcohol acyltransferase (LAAT) activity, which catalyzes the transfer of fatty acids in lecithin to the acyl moiety of wax esters, apparently is present in the plasma of carp (57) and could be responsible for at least a part of the plasma wax esters found in the species (30, 57).

A cholesteryl ester transfer activity has been found in trout plasma (155). It exchanges cholesteryl esters among the different plasma lipoproteins and is the most active known in vertebrates. This suggests the presence of a cholesteryl ester transfer protein (CETP) in the plasma of this species. As a result of this activity, there is little differ-

ence in the fatty acid composition of cholesteryl ester in the different classes of trout lipoprotein.

D. Origin and quantitative variations of plasma lipoproteins

The type and levels of the different classes of plasma lipoproteins in mammals are determined by a set of highly complex processes. They may be divided into different pathways for the metabolism of exogenous and endogenous fats and for the reverse transport of cholesterol from peripheral tissues to the liver (see refs. 125 and 156–159 for recent reviews).

Metabolic studies of plasma lipids and lipoproteins in fish remain limited. Nevertheless, existing data suggest that the processes and mechanisms involved in their metabolism have points in common with those of mammals.

1. Origin of plasma lipoproteins

In Agnatha, e.g., lampreys, feeding habits change during the life cycle. Larval forms (ammocoetes) filter debris and phytoplankton while adults either do not feed (nonparasite species) or consume blood, body fluids, or tissues of host fish (parasite species). During their anadromic reproduction migration, the animals cease feeding and their intestine and liver degenerate (40, 160, 161).

During the spontaneous feeding of adult *Petromyzon marinus,* an obligate sanguivore, VLDL accumulate in the hyaloplasm of the two types of intestinal absorbing cells, A and B, in the intercellular space, and the perivascular interstitial space of the lamina propria (162, 163). Type A cells are characteristic of the diverticulum and the proximal third of the anterior intestine, while type B cells exist only in the rest of the anterior intestine and in the transition zone preceding the posterior intestine. The role of A and B cells in lipid absorption and VLDL synthesis has been confirmed by a study of fasting sexually immature lampreys that had been force-fed polyunsaturated lipids (164). Two biological problems underlie lipid absorption in lampreys. First, adults lack bile canalicules and canals, and no exocrine route of bile transport to the intestinal lumen has been shown (165, 166). Secondly, the absence of a lymphatic system a priori is a barrier to the transport of intestinal lipoproteins to the rest of the body. The discontinuity of intestinal blood capillary walls (164, 167), however, and the absence of tight junctions between endothelial cells and their wall permit the uptake of lipoproteins formed by the absorbing cells, similar to what is observed in the lymphatic capillaries of other vertebrates. Lipoprotein particles are, in fact, present in the intestinal blood capillaries of lampreys during lipid force-feeding (164).

As in mammals, the liver in lampreys is a site of synthesis of plasma lipoproteins (168). The VLDL are a major component of Golgi elements and associated small vacuoles in the liver cells of parasite lampreys (166).

Lampreys carrying out reproduction migration have large quantities of plasma lipoproteins, VLDL, LDL, and HDL, even though they do not feed, as was shown in *Mordacia mordax* (40). Considering the progressive degeneration of the intestine and liver, what could be the origin of these lipoproteins? In the migrating arctic lamprey *Lampetra japonica,* proximal renal tubule cells secrete lipoprotein particles with the size of VLDL (169). These particles are discharged into the sinusoid capillaries of the subepithelial lamina propria via a process highly comparable to that described for VLDL synthesis by intestinal epithelial cell after feeding. During the migration period, the primary energy source is lipids and proteins of the body wall (40). Lipids account for 20 to 45% of dry body weight and decrease by 90% at the end of the reproduction period. Free fatty acids can be oxidized directly in muscle after lipolysis or pass into the plasma during anadromic migration (170, 171). The free plasma fatty acids can be used for VLDL synthesis by the proximal renal tubules (169). The notion that the kidney is capable of synthesizing lipoproteins is supported by recent findings showing apoB synthesis by this tissue in chicken (172, 173) and rabbit (174).

In teleost fish, intestinal absorption of fats and secretion of lipoproteins into the lymph by the intestine are basically comparable to the mammalian process, even though assimilation is slower in fish (see ref. 7 for review). The maximum of the absorption peak in trout is 18 to 24 hr after feeding (175, 176), in agreement with histological and biochemical observations (17, 29, 110, 177–182) carried out during study of the assimilation of lipids and lipid-soluble substances in teleost fish. Dietary lipids are absorbed in the anterior intestine and the pyloric caeca by species possessing them (175, 176, 181, 183–194) similarly to the first third of the mammalian intestine (see refs. 195 and 196 for reviews). Triglycerides and wax esters are the two main forms of neutral lipids available to fish in their natural environment (5, 47). In contrast to mammals, free fatty acids and glycerol are the major products of the luminal hydrolysis of dietary triglycerides in fish resulting from the nonspecificity of pancreatic lipase (197, 198) or the additional action of a β-monoglyceride lipase (199). Enzymatic hydrolysis and absorption of wax esters also occurs in the pyloric caeca and intestine of various species studied (197, 198, 200, 201) but the process is slower than that for triglycerides. Fatty acids are then reesterified in intestinal epithelial cells primarily as triglycerides (176, 179, 181, 202). Most of the fatty alcohols produced by the hydrolysis of wax esters are oxidized to their corresponding fatty acids in the intestinal tissue (203). They are then reesterified in place into acyl lipids, principally triglycerides and phospholipids, as shown in the gourami (203–205), trout (206), and carp (30). Dietary lipids are present in two forms in intestinal epithelial cells, stored in the form of large lipid droplets or exported as lipoproteins.

These particles have been observed under the electron microscope in the intestinal cells of trout (175, 176, 183, 185, 186, 207–210), carp (184, 188, 190), goldfish (187), tench (189), perch (191), catfish (193), and mullet (211). In the trout, for example, lipoproteins are present in the cisternae of the endoplasmic reticulum, in Golgi vesicles, inside lamellar structures, intercellular spaces, and interstitial spaces of the lamina propria and the lumen of lymph vessels (176). Obligatory passage via the Golgi apparatus does not necessarily appear to be a required step for the secretion of these lipoproteins (176, 210). Export of the particles apparently occurs only via the portal route in carp (188), only via the lymph in trout (176), or via both the tench and perch (189, 191). In all the species studied in which a standard diet was fed, it was noted that the size of these lipoproteins was closer to that of VLDL than the chylomicrons of mammals (175, 176, 188–190). In trout, however, the volume of lipoproteins depends on the dietary lipid load, the concentration of unsaturated fatty acids, and the degree of unsaturation of these fatty acids (208–210). The presence of chylomicrons in the plasma of this species has been shown in standard alimentary conditions (31, 52, 212–214). As a result of the complete and preferential luminal hydrolysis of dietary triglycerides with polyunsaturated fatty acids (194, 198, 199), the predominant route of intracellular esterification is that of glycerol-3-phosphate which leads to the biosynthesis of glycerophospholipids and triglycerides. In mammals, one of the major products of hydrolysis is 2-monoacylglycerol, whose esterification leads only to the synthesis of triglycerides. For the same supply of triglycerides, the synthesis of surface material, e.g., phospholipids, is greater in fish. Chylomicrons and VLDL of intestinal origin are thus smaller as are the low density lipoproteins in the plasma (see Table 4). In spite of the fact that nearly all fatty acids are reesterified in intestinal cells and are incorporated into newly formed lipoproteins, the direct passage of free fatty acids from the intestinal lumen into the circulatory system has been described (178, 179, 215). This process appears to occur during early absorption and under special alimentary conditions (4,192). Free fatty acids represent only a small proportion of total fatty acids carried by the blood (16, 171, 176) and their level is comparable to that observed in mammals and birds (171). They are probably combined in the blood with plasma fatty acid-binding proteins (216, 217) comparable to their binding to albumin in mammals.

During the development of fish, plasma VLDL may have different origins. Endogenous intestinal VLDL (218), hepatic VLDL (219–221), and VLDL synthesized by the yolk syncytial layer from yolk triglycerides (219, 222) are circulated in the embryo. After the first feeding, plasma VLDL of juveniles and then of adults have a double origin (13), as in mammals: intestinal as seen above, and also hepatic (220). In trout, the intestine and liver are thus major sites of apolipoprotein biosynthesis (223). Liver cells are also the site of vitellogenin biosynthesis, whose gene(s) can be induced by 17β-estradiol (136–138), resulting in ultrastructural changes in liver cells (71, 224–227) and substantial vitellogenin synthesis in female fish during vitellogenesis or in fish treated with estrogens.

2. Quantitative variations of plasma lipoproteins

Nearly all circulating lipids are present in plasma lipoproteins. There still exist relatively few data concerning the quantitative changes of different classes of fish lipoproteins, but a large body of work has shown that the plasma concentration of lipids, particularly cholesterol (see Table 2) and triglycerides in fish is highly dependent on their nutritional or physiological state and on their developmental stage. For example, in the lamprey *Petromyzon marinus,* the characterization of a lipoprotein called CB-III (39) has shown that its concentration in the plasma varied with the different phases of the life of the animal (228).

Factors such as age and growth, sex, temperature, salinity, or certain endocrine factors can affect plasma cholesterol levels in fish (see refs. 7 and 229 for reviews). Above all, it is alimentation and the nutritional state and/or the reproduction cycle that have a profound influence on the concentration of plasma lipids. There exist considerable seasonal changes in cholesterol concentrations related to the reproductive cycle, the lowest levels generally being recorded during sexual maturation and egg laying.

Some fish continue feeding during genital maturation, but others undergo an appetite reduction during spawning or cease feeding altogether shortly before and during spawning or long before as is the case for salmon and eels. In the latter case, fasting is associated with reproduction migration which may be very long. In the course of an experimental fast or a natural one during spawning migration, lipids are mobilized from reserve tissues: liver, skeletal muscle, and in some species visceral adipose tissue, not only to be used as energy source, but also to insure the formation of genital products (see refs 7 and 62 for reviews). In addition to vitellogenin uptake (131), the ovary is apparently capable of taking up lipid components through the lipolysis of circulating lipoproteins. In trout, this is revealed by a substantial increase in lipoprotein lipase and salt-resistant lipase activities in the ovary during exogenous vitellogenesis (147).

Plasma lipoproteins are a dynamic system responding to food intake (17, 29, 123, 178), to its frequency, and to the nature of the ingested lipids in the diet. In the course of an experimental fast in catfish (17, 123) or trout (149), the levels of VLDL and LDL decreased sharply, while that of HDL remained unchanged even after a very long period without eating. In agreement with the mammalian model, experiments in catfish have suggested that much of

the plasma LDL is derived from sequential lipolysis of triglyceride-rich VLDL to IDL and then LDL (123). During the spawning migration of the salmon *Oncorhynchus gorbuscha,* the animals stop eating and plasma levels of triglycerides decrease substantially (230). At this time, the liver cannot synthesize triglycerides (231), which would explain the absence of VLDL and LDL in the plasma just before egg laying (37). The concentration of HDL at this time may be as high as 3,300 mg/dl of plasma (37), much different from the 238 mg/dl observed in young salmon *Oncorhynchus nerka* (111).

In trout, the quantitative variations of different lipoprotein classes are related primarily to age (212), but also to the stage of sexual maturity (27, 69, 147). In young fish, LDL predominate, while in adults HDL are the major form; a similar situation exists during the development of some other vertebrates (114, 232). In adults, there are seasonal variations in the levels of various plasma lipoprotein classes throughout the annual reproduction cycle. VLDL, LDL, and HDL are more abundant in females after egg laying than during the preceding months (69, 147). Changes in plasma lipoprotein concentrations may be the reason for changes in plasma LCAT activity observed during sexual maturity or fasting in carp (151), char (233, 234), and trout (147). These lower concentrations in sexually mature animals result in decreased plasma concentration of triglycerides (147) and also in cholesterol (see Table 2), in spite of the concomitant increase in the level of plasma vitellogenin in females (2% by weight in cholesterol, see Table 5). The considerable seasonal variations in the plasma levels of vitellogenin observed in teleost fish (see Table 3) are associated with the circulating level of 17β-estradiol (85, 87, 235–239). Circulating cholesterol of plasma lipoproteins is the main source for synthesis of this steroid (214, 240, 241). The presence of reversible fibrous lesions of coronary atherosclerosis in fish (see ref. 54 for a review) related to the reproductive cycle may be due to these quantitative changes in plasma lipoproteins and/or sex hormones. These animals may constitute a valuable model for the study of certain hormonal and nutritional factors that initiate atherosclerosis.

Note added in proof: Subsequent to submission of this manuscript, additional data appeared in the literature describing an immunological cross-reactivity between the major apolipoproteins (A-I and A-II) of trout and human HDL (278). Other results (279) demonstrated that carp VLDL and LDL competed with the specific binding of human LDL to human fibroblast LDL receptors. Carp liver lipoprotein receptors have also been described similar in their specificity to human and other mammals.

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