review

Animal lipoproteins: chemistry, structure, and comparative aspects[1]

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OVERVIEW

Although use of plasma from the horse (Equus sp.) facilitated the earliest isolation of a soluble lipoprotein of constant composition by Macheboeuf in 1929 (1), subsequent attention has centered upon the human lipoprotein system; numerous reviews in recent years attest to this (2–4). Such a situation is a reflection of man's susceptibility to cardiovascular disease and of the implication of the serum lipoproteins in its development.

Knowledge of lipid transport systems in animals has advanced in recent years, particularly as a result of the extensive use of a number of higher animal species as experimental models for lipid and lipoprotein metabolism in man, albeit often under conditions of atherogenic diet. Some progress in this sphere is also due to the ever-increasing importance of foodstuffs of animal origin in human nutrition, which has in turn incited basic research into the nutrition of animals themselves and into their modes of lipid absorption and transport. It is pertinent to remark therefore that there is a paucity of data on the quantitative and qualitative characteristics of the major lipoproteins and apolipoproteins in a number of commonly-used species under normal dietary conditions. Moreover, data on animal lipoproteins are often disperse, although information up to 1975 has been reviewed by Mills (5). In addition, data are commonly subject to

Abbreviations: Apo, apolipoprotein; VLDL, very low density lipoproteins of d < 1.006 g/ml, unless otherwise defined; IDL, intermediate density lipoproteins, of d 1.006–1.019 g/ml, unless otherwise defined; LDL, low density lipoproteins, density as defined; HDL, high density lipoproteins, of d 1.063–1.21 g/ml, unless otherwise defined; VHDL, very high density lipoproteins, density as defined; LCAT, lecithin:cholesterol acyltransferase; SDS, sodium dodecyl sulphate.

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[1] No reprints available.

the limitation that lipoprotein fractions were prepared after tacit application of criteria developed specifically for the isolation of human lipoproteins (e.g., density intervals for ultracentrifugal flotation). Such a limitation often concerns the boundary between the low density and high density lipoproteins, (i.e., 1.063 g/ml) and may lead to an incomplete separation of these substances as first noted by Mills and Taylaur (6).

The need for a comprehensive review of the chemistry and structure of animal serum lipoproteins is thus evident, not only for the critical assessment of older information but also for collation with that which has appeared more recently. The present review represents an attempt to fulfill this requirement, and is based on the phylogeny of the animal kingdom. Such an approach might be expected to reveal the nature of the evolution of the serum lipoproteins as a function of the complexity of the parent organism.

PRELIMINARY CONSIDERATIONS

Throughout, reference will be made to the human serum lipoprotein system as a standard whenever appropriate. Human serum apolipoproteins, as well as any recognized animal counterpart, will be designated according to the nomenclature of Alaupovic (7). Isolation of lipoproteins from animal sera or plasma has been performed partially or wholly on the basis of hydrated density by the majority of investigators; such an approach necessarily delimits the final lipoprotein fraction in rather precise terms and facilitates exact reproduction of experimental conditions in different laboratories. Although lipoproteins will de defined primarily with respect to the hydrated density limits within which they were isolated, other designations (notably electrophoretic mobility) will be mentioned when useful.

The designation of animal lipoproteins on the basis of both their hydrated density and apolipoprotein content is of course ultimately desirable; indeed, such a nomenclature could be derived from the lipoprotein family classification proposed for the human plasma lipoproteins (7), that has its basis in apolipoprotein composition. However, our elementary knowledge of apolipoproteins in all but a few animal species, considered together with difficulties in assigning homologous relationships to a given pair of animal and human polypeptides, currently precludes this approach. Nonetheless, the recent identification of protein components analogous to the human A (essentially A-I), B, and arginine-rich apolipoproteins in a variety of vertebrates, including species of fish, reptiles, birds, and mammals, provides a framework for future progress along these lines.

Any discussion of animal serum lipoproteins would be incomplete without addressing some of the rather specific problems which one may meet in their study. Thus, the background density (i.e., density exclusive of macromolecule content) of animal serum, an important parameter when lipoproteins are isolated by ultracentrifugal flotation, may not be identical to that of man (i.e., 1.006–1.007 g/ml). In the case of fish sera (both Elasmobranchs and Teleosts), for example, the background solvent densities are higher and may vary from 1.015 to 1.025 g/ml (8, 9), while that of equine serum (10) is rather lower (1.004 g/ml). Moreover, the frozen storage of animal sera, often desirable as a result of the inaccessibility of certain species and of consequent sample transport, may also involve alteration of background density (10). The determination of this parameter, by simple equilibrium dialysis or ultrafiltration techniques (10), therefore appears an important prerequisite to fractionation of animal lipoproteins, especially when hydrated density is employed as the primary criterion for isolation.

The various methods of lipoprotein separation also deserve some general consideration, since the limitations and possible artifactual effects of each should be borne in mind. Thus, repetitive ultracentrifugal flotation, commonly employed in the separation of animal serum lipoproteins, leads to irreversible structural and compositional (lipid and protein components) alterations in human VLDL (11), and to loss of apoprotein A-I from human HDL (12). Such effects are not confined to the human substances, since use of fixed angle rotors generating high centrifugal forces during flotation of rat serum lipoproteins leads to progressive loss of the arginine-rich apolipoprotein (i.e., apolipoprotein E in Alaupovic's nomenclature (7)) into the bottom ($d > 1.21$ g/ml) fraction (13). This latter phenomenon has been confirmed by comparison of the apoprotein content of rat lipoproteins prepared by gel filtration chromatography on 10% agarose and by ultracentrifugal flotation (14), which revealed substantially reduced concentrations (~30–60%) of apo-E in ultracentrifugally-isolated VLDL and HDL. Such effects may have their origin both in the centrifugal force applied to the lipoproteins, and in their exposure to high salt concentrations. The importance of these factors, as well as the geometry of the ultracentrifugal separation, in determining the composition of the final product has been further emphasized in a study of Cebus (capuchin) monkey lipoproteins (15). Thus, VLDL isolated in a fixed angle rotor was richer in phospholipid and pro-

tein, and poorer in cholesterol and triglyceride, than the VLDL isolated by density gradient centrifugation.

An additional critical parameter in the centrifugal fractionation of animal sera is the temperature at which the separation is performed, since the rate of thermal expansivity of serum LDL in particular is higher than that of the aqueous media in which it is normally isolated (16, 17); the differential effect of temperature is essentially negligible in the case of HDL due to the low temperature coefficient of dense NaCl/NaBr solutions. Isolation of LDL should be performed preferably at the same temperature as that at which determination of solvent density was carried out (often 20°C); failing this, data on the composition and density of the solvent at a given temperature should be quoted, together with the temperature of centrifugation.

Since the above criticisms concern problems associated with the separation of serum lipoproteins by ultracentrifugal flotation, it would be misleading to omit comment on alternative procedures. Thus gel filtration chromatography on agarose columns permits a satisfactory separation of the major lipoprotein classes (e.g., from rabbit serum (18)), but only after their ultracentrifugal flotation at d 1.21 g/ml; the problem of exposure to high ionic strength and centrifugal force is not therefore circumvented. Direct application of serum to such columns minimizes introduction of artifacts and, as in the case of the rat, has allowed fractionation of VLDL, LDL, and HDL, although HDL was incompletely separated from other serum proteins (14).

Additional parameters of importance in the study of animal lipoproteins involve the strain of the species in question, and the age and sex of the individuals. The latter is particularly relevant since female-specific lipoproteins typically occur during vitellogenesis in both invertebrates and vertebrates. The conditions under which the blood samples were withdrawn are also of significance, and include the nutritional state of the animal (fed or fasting), its diet, the nature of its environment (i.e., captive or wild) and in certain cases, the temperature at which it was maintained. In the ensuing review, such information as is pertinent to the parameters discussed above will be noted whenever possible.

INVERTEBRATE LIPOPROTEINS

As is evident from **Fig. 1,** data on the soluble lipoproteins of species belonging to that vast part of the animal kingdom constituted by the invertebrates is limited to members of the phylum Arthropoda. Indeed, the only investigations of which the author is aware concern two of the four classes of arthropods, namely crustaceans and insects.

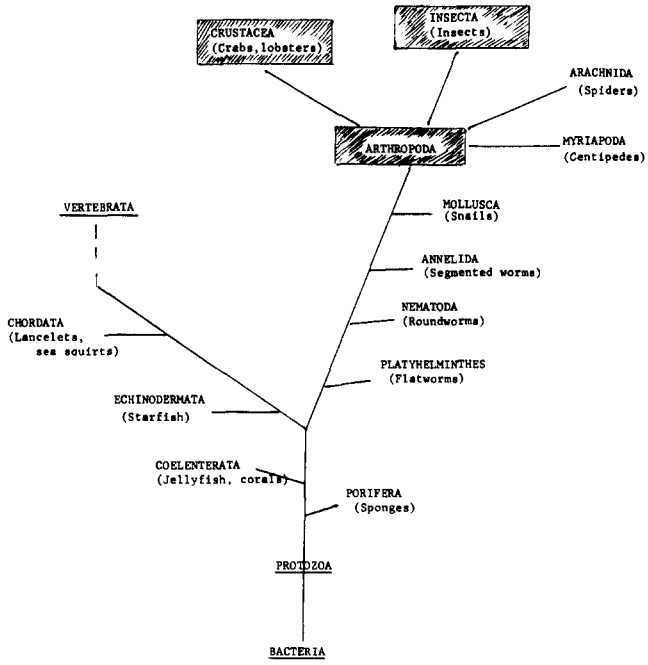

Fig. 1. Diagrammatic representation of a simplified family tree of the animal kingdom, showing the approximate relationships of the principal inverterbrate phyla and including the chordate ancestors. Phyla and classes for which data on lipid transport systems are available are represented in shaded boxes.

Crustaceans

Unfortunately, simple and inadequate methods, (such as the detection of colored bands following electrophoresis of hemolymph and gel-staining with lipid-soluble dyes), were often used in investigations of the hemolymph lipoproteins of decapod crustaceans (19–24). Up to three lipoprotein species could be resolved in this way in the hemolymph of male and female specimens of the Dungeness crab (*Cancer magister* Dana) (19) and of the crab *Paratelphusa hydrodomous* (23); at least one form was limited to females. Female-specific lipoproteins have also been identified in the fiddler crab (*Uca pugilator*) (20), in the shore crab (*Carcinus maenas*) (22) and in the blue crab (*Callinectes sapidus*) (21); in all of these instances, the occurrence of such macromolecules was intimately related to ovogenesis (i.e., ovarian maturation). Furthermore, the hemolymph lipoprotein of the female blue crab, isolated by paper curtain electrophoresis, was found to be identical with the purified high density oocyte lipoprotein (d < 1.4 g/ml) upon immunodiffusion analyses using an antiserum to the oocyte frac-

tion, and to display the same low electrophoretic mobility (21). The yellowish color of the hemolymph lipoprotein, noted in this (21) and the other studies (20, 22), is apparently due to its content of carotenoids. Indeed, since the maturing oocytes of *C. sapidus* contained large amounts of β-carotene, and since the hemolymph and egg lipoprotein appeared to possess common protein moieties, Kerr (21) was led to suggest that the hemolymph lipoprotein was a precursor to its egg yolk counterpart, being incorporated into the latter by the oocytes during vitellogenesis. On the basis of its physical properties and lipid content (27.5%), the egg lipoprotein of the blue crab was termed a 'lipovitellin' (21), in keeping with the analogy observed in crustacean egg lipoproteins and lipovitellins isolated as high density lipoproteins from vertebrate eggs by Wallace, Walker, and Hauschka (25).

Female-specific lipoproteins may also occur in species of lobster, as the data of Barlow and Ridgway (26) suggest in *Homarus americanus*; the question as to whether the high density lipoprotein isolated from spiny lobster (*Panulirus interruptus*) hemolymph (24) is equivalent was not addressed. This latter report appears unique in that the high density lipoprotein species was isolated as an HDL_3 (d 1.12–1.21 g/ml) fraction by ultracentrifugal flotation; it contained 45–50% lipid and accounted for essentially all of the hemolymph lipids. Of these, the major class was phospholipids (88% of the total), most of which were phosphatidylcholine. Small amounts of free sterol and triglycerides (3–4%), together with traces (1% or less) of hydrocarbons, free fatty acids, cholesteryl esters, and diglycerides were also detected. The predominant fatty acids in the triglycerides and phospholipids were 16:0, 16:1, 18:0, 18:1, and 20:5 in the former, and 16:0, 18:0, 18:1, 20:5, and 20:6 in the latter; the degree of unsaturation being slightly higher in the phospholipids (68%) as compared to the triglycerides (64%).

These data are in overall agreement with those obtained by Allen (19) in exhaustive lipid analyses of hemolymph from male and female Dungeness crabs. Although phospholipids (primarily phosphatidylcholine) again predominated (an average of 65% of the total) in both sexes, the proportions of cholesterol (10.4–13.5%), diglycerides (8.9–11.0%), triglycerides (1.7–6.7%), and of hydrocarbons and wax esters (2.3–6.7%) were higher in *C. magister* (19) than in *P. interruptus* (24). Cholesteryl esters and free fatty acids were present in trace quantities, as were diacylglycerylethers (<1% of total). Interestingly, the major fatty acids of *C. magister's* phospholipids and triglycerides were essentially identical with those noted above for *P. interruptus*, with the exception that the triglycerides of the former were more highly unsaturated (70%). While the fatty acid profiles of the diglycerides and triglycerides of Dungeness crab hemolymph were shared, that of its free fatty acids was distinguished by an elevated content of the long-chain polyene 20:4(n-6). The protein moieties of crustacean hemolymph proteins have been only superficially investigated, but the preliminary findings suggest they may resemble the major apoprotein of human LDL, i.e., apolipoprotein B, in solubility properties (21, 24). Thus, dissolution of the delipidated apoprotein of the hemolymph lipoprotein of the blue crab (21) was accomplished only in detergent (SDS) solution. Significantly, this apoprotein was specific to the lipoprotein and distinct from other hemolymph proteins.

Although cursory, our current knowledge of lipid transport in Crustaceans would appear to indicate that the functional molecules are relatively complex lipoprotein species, which contain specialized lipid-binding protein(s). These hemolymph lipoproteins appear to be primarily high density in nature and to fulfill a dual role, i.e., the transport of pigments (especially carotenoids), lipids, and protein to the maturing oocytes during vitellogenesis in females, and the transport of lipids (presumably as diglycerides and triglycerides) as a source of energy in both sexes. Some support for the latter suggestion may be derived from Allen's (21) observation that hemolymph diglyceride levels are unrelated to sex or reproductive status in *C. magister*, although the low levels of diglyceride and more "primitive" neutral lipids (i.e., hydrocarbons and wax esters) in the HDL_3 of the spiny lobster appear in contradiction of this hypothesis.

Insects

Without doubt, diglyceride functions as the primary form in which lipid is transported in insects (27), this group being the most extensively studied of the invertebrates. Lipids of various types are in fact implicated in a myriad of processes in these arthropods, among which are locomotion, reproduction, embryogenesis and metamorphosis; they also function as hormones in metabolic regulation and as structural components. However, although there is substantial information on the role of hemolymph lipoproteins in diglyceride release from the insect fat body and in sterol and hormone transport (27), data on the structure of the macromolecules themselves are scant. Detailed chemical information is essentially restricted to two species of saturniid silkmoth, *Hyalophora cecropia* and *Philosamia cynthia*, to the locust, *Locusta migratoria*, and to the tobacco hornworm, *Manduca sexta.*

Nonetheless, soluble lipoproteins have been de-

tected in a wide range of insects, often upon the basis of their specific (lipid) staining following hemolymph electrophoresis in various media. These species include other Lepidoptera, (*Hyalophora gloveri*, *Callosamia promethea*, *Antheraea polyphemus*, *A. mylitta*) (28), the grasshopper (*Melanoplus differentialis*) (29), the American cockroach (*Periplaneta americana*) (29, 30), the Colorado potato beetle (*Leptinotarsa decemlineata*) (31), the fruit fly (*Drosophila hydei*) (32), and the housefly (*Musca domestica*) (33). Several lipoprotein bands (from four to seven) were detected in the silkmoths (28), the Colorado potato beetle (31), and the American cockroach (30). In all of the saturniids examined (28), the lipoproteins uniformly possessed a carbohydrate moiety, but whether lipoprotein-carbohydrate may make a significant contribution to overall hemolymph sugar content (34) awaits assessment.

In a similar manner to that noted in the marine crustaceans discussed above, a major female-specific lipoprotein was detectable in the hemolymph (larval, pupal, and adult) of several of the Lepidopteran species examined by Whitmore and Gilbert (28). Subsequent comparison of the electrophoretic profiles given by their hemolymph and egg proteins showed the major egg protein to be a lipoprotein with an electrophoretic mobility identical to that of the "female" hemolymph lipoprotein in each insect. A further analogy with the Crustacea was seen in these two lipoproteins, (each of which was present in one or more forms according to the genus of insect), since they were yellow in color and accounted for the pigmentation of the egg and hemolymph respectively. Such findings prompted the authors to suggest that the hemolymph lipoprotein corresponded to a "sex-limited" protein, i.e., a vitellogenin.

Whereas the earliest characterizations of the two principal forms of hemolymph lipoproteins in the saturniids *Hyalophora cecropia* (35) and *Philosamia cynthia* (36) were accomplished in the late 1960's, it was not until comparatively recently that the physicochemical identity of one of these lipoproteins (in *P. cynthia*) with the corresponding egg vitellogenin was demonstrated (37, 38). The 'vitellogenic' hemolymph lipoprotein was originally isolated as diglyceride-carrying lipoprotein of Type II by Chino, Murakami, and Harashima (36), subsequently referred to as LP-II (27). Regrettably, considerable confusion is evident in the literature in the terminology of different insect hemolymph lipoproteins, and this appears to have arisen as a result of the various techniques employed in their separation. For present purposes, lipoprotein fractions will be designated on the basis of their hydrated density, primarily to permit comparison with the human lipoproteins; this does not imply, of course, that the insect hemolymph and mammalian serum lipoproteins are analogous in function. The exemplary study is that of Thomas and Gilbert (35), who isolated and characterized three classes of hemolymph lipoproteins from pupae of the American silkmoth, *Hyalophora cecropia*. These substances were separated in density intervals approximating those of the human lipoproteins and were low density lipoproteins of d 1.046–1.063 g/ml, high density lipoproteins of d 1.156–1.17 g/ml, and very high density lipoproteins of d < 1.26 g/ml; chylomicrons and VLDL were undetectable. Although the hemolymph lipoproteins LP-I and LP-II of *P. cynthia* were isolated by a procedure involving ammonium sulfate fractionation, precipitation at low ionic strength, and ion-exchange chromatography (36), evaluation of their chemical composition clearly indicated LP-I to be an HDL species and LP-II a VHDL (27).

By use of density gradient ultracentrifugation and gel filtration chromatographic techniques, high density and very high density lipoprotein particles have also been isolated from the hemolymph of the tobacco hornworm, *Manduca sexta* (39, 40). HDL was separated from fifth instar larvae and displayed a hydrated density of 1.15 g/ml; VHDL, (density 1.29 g/ml) represented a vitellogenin, and was specific to adult females. As might be anticipated, the molecular weight of *M. sexta* HDL was greater than that of its vitellogenin or VHDL (6×10^5 and 2.6×10^5, respectively). Designation of the lipoprotein characterized from locust (*Locusta migratoria*) hemolymph (41) is more tentative, but its chemical and physical properties suggest it to be a form of LP-I or HDL. The two groups of lipoproteins isolated from the larval hemolymph of houseflies (*Musca domestica*) by Dwivedy and Bridges (33) were termed β- and α-lipoproteins on the basis of their migration similar to that of human lipoproteins on agarose gel, and it was suggested that they were LDL and HDL, respectively. Their classification is based at best on a single and tenuous criterion.

Gamo (42) could identify three or more groups of lipoproteins by electrophoresis in the pharate adult hemolymph of the silkworm *Bombyx mori*. Although stainable with Sudan Black B, the lipid content of these lipoproteins was not determined. Their relationship to the human compounds is thus indeterminate. After separation by a combination of precipitation, gel filtration, and ion-exchange chromatographic procedures, the three major species were found to display remarkably low molecular weights (19,200–21,000). They are therefore substantially smaller complexes than those typical of the hemo-

TABLE 1. Chemical compositions of insect hemolymph and human serum lipoproteins

	LDL		HDL					VHDL				
	Hyalophora cecropia[a] (Silkmoth)	Man[b]	*H. cecropia*[a]	*Philosamia cynthia*[c] (Silkmoth)	*Locusta migratoria*[d] (Locust)	*Manduca sexta*[e] (Tobacco hornworm)	Man[f]	*H. cecropia*[a]	*M. sexta*[g]	*P. cynthia*[c]	Man[h] VHDL$_1$	Man[h] VHDL$_2$
Density (g/ml)	1.046–1.063	1.040–1.063	1.158–1.170			1.15	1.063–1.21	<1.26	1.29		1.210–1.250	>1.250
					% (by weight)							
Nonesterified fatty acids	1.6		1.3		1.5	1.0	0.9	0.4			0.6	0.6
Sterol esters	4.4	38.0	2.0			(5.0)*	14.7	0.2			3.2	0.04
Sterols	8.9	7.9	2.6	5.8	2.3	2.0	2.8	0.9	5.8	1.2	0.3	0.02
Triglycerides	8.0	5.0	2.9	0.5	0.6	2.3	1.9	0.5				
Diglycerides	33.4		27.2	24.8	13.1	12.6	0.4	2.0		3.3	4.7	0.04
Monoglycerides	2.1		1.0		1.8	0.2	0.3	0.2				
Hydrocarbons	9.3		2.4			(5.0)*	0.4	0.3				
Phospholipids	8.4	22.7	8.6	11.4	12.1	14.0	17.7	1.7	5.6	4.8	28.8	0.8
Protein	24.0	26.3	52.0	56.0	68.5	61.0	57.7	94.0	86.0	90.3	62.4	98.5

[a] Fractions lacking density limits were isolated by alternative procedures to ultracentrifugal flotation. Calculated from the data of Thomas and Gilbert (35).

[b] The mean composition of LDL subfractions V and VI from normal human plasma (43).

[c] Calculated from the data of Chino et al. (36); monoglycerides and sterol esters not detectable.

[d] The mean composition of two separate preparations of each fraction (41); monoglycerides possibly present due to diglyceride hydrolysis. Cholesteryl ester not detectable.

[e] Calculated from ref. 39. *Hydrocarbons and sterol esters determined together. Carbohydrate represented 2% by weight.

[f] Data from Skipski et al. (44); unidentified lipids (1.0%) and glycolipids (0.2%) excluded.

[g] From ref. 40. Neutral lipids identified individually by thin-layer chromatography, but quantitated together. Sterol esters absent. Carbohydrates (mannose and *N*-acetyl glucosamine) represented 3% by weight.

[h] Data from Alaupovic et al. (45).

lymphs of the saturniid silkmoths (35–37), locust (41, 50), and tobacco hornworm (39, 40), and may at most represent protein-fatty acid complexes.

The chemical compositions of these various insect lipoproteins are compared to each other and to those of the corresponding human lipoproteins in **Table 1.** Insect LDL is clearly distinct in both structure and function from that of man, since diglycerides are its major lipid, whereas cholesteryl ester and phospholipid predominate in human LDL. Indeed the silkworm LDL would appear implicated in the transport of diglyceride in a similar manner to the HDL (LP-I) fraction (29), although in a minor way since it represented only about 5% of the total pupal hemolymph lipoproteins in *H. cecropia* (35). The elevated hydrocarbon content of the hemolymph LDL is particularly notable, especially since these compounds form the bulk of the cuticular lipids in all the adult insects analyzed so far (46); they consist of n-alkanes, unsaturated hydrocarbons, terminally-branched 2-methyl and 3-methyl alkanes and internally-branched monomethyl-, dimethyl- and trimethylalkanes (47).

The HDL fraction, a major component of *H. cecropia* (43% of the total lipoproteins) and *L. migratoria* hemolymphs, was rich in diglyceride (13–27%) in all four insects examined. Substantial evidence has been provided for the role of this class of lipoprotein in lipid release (as diglyceride) and transport from the fat body to its sites of utilization (29), one of which is the ovary (48). Furthermore, several authors have suggested that the chemical composition of this lipoprotein may vary according to the physiological state of the fat body (29, 41), and the variability seen in certain of the physical properties of *L. migratoria* HDL is entirely consistent with this possibility (41). Despite an overall resemblance in chemical composition, some differences were seen in the insect HDLs (Table 1), notably the lack of hydrocarbons and sterol esters in the *P. cynthia* and *L. migratoria* fractions. While the proportions of protein and of phospholipid were alike in insect and human HDL, those of the remaining lipids were quite distinct, almost certainly reflecting the distinct roles of this lipoprotein class in the two species.

The silkworm and tobacco hornworm lipoproteins of very high density were similarly enriched in protein; the content was more than 85%. However the lipid moiety of *P. cynthia* VHDL was constituted by sterols and diglycerides, whereas that of *H. cecropia* and *M. sexta* contained other glycerides, sterol esters (the former only), hydrocarbons, and free fatty acids in addition. The insect VHDL fractions showed essentially no resemblance to either human VHDL$_1$ or VHDL$_2$.

TABLE 2. Weight percent distribution of phospholipids in insect and human lipoproteins

	LDL			HDL					VHDL			
	Hyalophora cecropia[a] (Silkmoth)	*Musca domestica*[b] (Housefly)	Man[c]	*H. cecropia*[a]	*Philosamia cynthia*[d] (Silkmoth)	*M. domestica*[b]	*Manduca sexta*[e] (Tobacco hornworm)	Man[c]	*H. cecropia*[a]	*P. cynthia*[d]	*M. sexta*[f] (Vitellogenin)	Man[g] $VHDL_2$
Phosphatidylcholine	44.9	8.8	63.7	47.3	48	8.5	32.9	74.4	49.4	44	22.9	28.1
Sphingomyelin	21.1		25.9	19.4	20		10.8	13.2	12.8	18	0.5	7.7
Phosphatidylethanolamine	21.5	50.5	2.2	23.8	32	51.2	52.1	3.1	27.8	38	71.0	
Phosphatidylserine	11.1	10.0	0.8	9.5		16.0		0.8	6.7			
Phosphatidylinositol			1.6					2.4				
Lysolecithin		1.0	2.7			1.8	trace	2.9				64.1
Phosphatidic acid and polyglycerophosphatides	1.3	3.7	2.0	1.8		2.6		2.2	3.0			

[a] Data from Thomas and Gilbert (35).
[b] Data on the housefly are from Dwivedy and Bridges (33); lipoprotein fractions tentatively identified as LDL and HDL on basis of electrophoretic migration. The proportions of ceramide, phosphorylethanolamine, phosphatidylglycerol, and unknown phospholipids have been omitted. Animals were fed diets containing choline.
[c] Data from Skipski et al. (44); unidentified phospholipids 0.9%.
[d] From Chino et al. (36).
[e] Calculated from Pattnaik et al. (39); lysophosphatidylethanolamine amounted to 2.9% of total.
[f] From Mundall and Law (40); lysophosphatidylethanolamine 5.6% by weight.
[g] Data from Alaupovic et al. (45).

The distribution of phospholipids in the silkworm, housefly, and tobacco hornworm hemolymph lipoproteins is shown in **Table 2,** and compared to the corresponding patterns in man. It is immediately apparent that the insect fractions are highly enriched in phosphatidylethanolamine. Peled and Tietz (41) also noted the presence of this lipid as a principal component in locust HDL phospholipid, which contained lecithin and phosphatidylethanolamine in a molar ratio of 7:1. The absence of sphingomyelin from the housefly lipoproteins is significant, as is the low level of lecithin. Furthermore, the LDL fraction was associated with more than 60% of the total phospholipid in housefly hemolymph. If indeed one of the functions of hemolymph lipoproteins is the transport of phospholipid (49), then marked dissimilarities may exist between different insect species in the manner in which this is effected.

The fatty acid distributions of the diglycerides and phospholipids of the insect lipoproteins are given in **Table 3.** In all of the silkworm and locust lipoproteins, the two classes of lipids contained palmitic and oleic acids as major components (20–56%). *P. cynthia* diglycerides were however distinguishable from those of *L. migratoria* by their elevated linolenic acid contents; conversely the locust phospholipids were distinct in their high proportions of stearic and linoleic acids (~10% or more). Fatty acids with

TABLE 3. Fatty acid compositions of phospholipids and diglycerides from insect hemolymph lipoproteins

Species	Lipoprotein Fraction	Lipid	Fatty Acid (% by weight)							
			12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Philosamia cynthia[a] (Silkmoth)	HDL	Diglyceride	2.1	0.2	29.0	1.8	1.1	25.8	3.7	36.3
Locusta migratoria[b] (Locust)	HDL	Diglyceride			55.6	trace	5.9	32.5	4.0	2.0
P. cynthia[a]	VHDL	Diglyceride	0.3	0.4	23.0	2.5	1.5	20.5	5.0	46.8
L. migratoria[b]	HDL	Phosphatidylcholine			39.4	trace	14.4	33.8	9.2	3.2
L. migratoria[b]	HDL	Phosphatidylethanolamine			27.6	5.8	18.2	24.2	15.5	8.7

[a] Data from Chino et al. (36).
[b] Data from Peled and Tietz (41).

more than 18 carbon atoms do not appear in these insect lipoproteins.

The protein moieties of insect lipoproteins have received little attention. Amino acid analyses of the delipidated apoproteins of the HDL and VHDL of *P. cynthia* and *M. sexta* have been reported (36, 39, 40), as has that of locust HDL (41). The similarity in amino acid profile of the silkworm, locust, and tobacco hornworm HDL apoproteins is noteworthy, each containing high levels of aspartic and glutamic acids, lysine, serine, valine, and leucine. The amino acid composition of *P. cynthia* VHDL was more distinct however, possessing lower contents of lysine and leucine, a higher proportion of glutamic acid, and an inverse ratio of tyrosine to phenylalanine (tyr:phe, 0.6 in *P. cynthia* HDL and 1.3 in VHDL). The apoprotein of *P. cynthia* VHDL, i.e., hemolymph vitellogenin, has recently been found to be a glycoprotein containing 2.5% mannose by weight (38); mannose is also a constituent of apo-HDL in this silkworm, but is present in slightly lower amount (1.4% by weight) (48).

The nature of the protein moieties of silkworm VHDL, locust and tobacco hornworm HDL and VHDL (vitellogenin) has been further evaluated by SDS-polyacrylamide gel electrophoresis. Locust hemolymph apo-HDL showed several bands whose molecular weights were not estimated (41). The apoprotein of its VHDL is also complex, presenting about eight components falling into two molecular weight groups, 140,000–105,000 and 65,000–52,000 (50). Silkworm apo-VHDL was simpler, being resolved into two major bands, with molecular weights of 230,000 and 55,000, and present in a 4:1 ratio as judged by densitometry (38). On this basis, the authors proposed that hemolymph VHDL in *P. cynthia* contains two of each of these subunits, giving a total molecular weight of 570,000. Taking into account the possible errors in molecular weight estimation in SDS gels (5–10%), and the additional weight due to lipid (10% by weight), a molecular weight of about 600,000 would be anticipated for *P. cynthia* VHDL. This is somewhat higher than the value of 504,000 originally reported by Chino et al. (36), and substantially more than that of 108,000 determined for *H. cecropia* VHDL by the same method (35). Similar discrepancies exist between the molecular weights of the HDL particles isolated from *P. cynthia*, *H. cecropia*, and *L. migratoria* hemolymphs, which have been determined as 690,000 (36), 189,000 (35) and 340,000 (41), respectively. These variations are not accounted for by dissimilarities in partial specific volume estimations, since these were rather similar in HDL (0.892, 0.87, and 0.84 in *H. cecropia* (35), *P. cynthia* (36), and *L. migratoria* (41), respectively). Values for VHDL differed more however, 0.831 and 0.75 in Hyalophora (35) and Philosamia (36), respectively.

Estimation of the molecular weight of the two major polypeptides (AL_1 and AL_2, 2.85×10^5 and 8.1×10^4, respectively) of tobacco hornworm apo-HDL correlated well with that of the particle (6.0×10^5) when a protein content of 61% is considered (39). These polypeptides appear present in a 1:1 molar ratio and are the only insect apolipoproteins to have been purified to date. Their amino acid compositions are indistinguishable. *M. sexta* apo-VHDL (40) is also made up of two distinct polypeptide subunits, AV_1 and AV_2, of lower molecular weight, 1.8×10^5 and 5×10^4, respectively, than their counterparts in apo-HDL. As in the latter apoprotein, one of each of these subunits is present per VHDL particle, whose molecular weight is 2.6×10^5 (40).

An attractive hypothesis linking the structural features of several insect vitellogenins, (of either hemolymph or egg yolk origin), has recently been propounded by Mundall and Law (40). In view of similarities in protein content (approx 90%; see Table 1), the glycoprotein nature of the apoproteins, the molecular weight of the native particles, and the number of polypeptide chains, these authors suggest that insect vitellogenins may have been derived from some simple form, such as that represented by the particles typical of *M. sexta* (40), *H. cecropia* (51), and *P. cynthia* (38). Accordingly, the complexity of the apoprotein of certain vitellogenins, such as that of *L. migratoria* (41, 50), was proposed to have arisen by proteolytic cleavage of a basic precursor (40). Chen, Strahlendorf, and Wyatt (50) have described direct evidence for such a posttranslational modification (involving both proteolysis and aggregation) of high molecular weight polypeptide precursors (265,000 and 250,000) prior to their secretion (as vitellogenin) from fat body cells in the locust. Further data on the apoproteins of insect vitellogenins (i.e., VHDL) are however required in order to assess whether this phenomenon occurs more widely.

While the various hemolymph lipoproteins discussed above were each judged homogeneous on the basis of ultracentrifugal and electrophoretic criteria, some of them presented several bands upon disc gel electrophoresis, such as in *H. cecropia* LDL, HDL and VHDL (35). The significance of these bands and their relationship to the multiple lipoprotein species typically detectable in whole hemolymph warrants further investigation.

The yellow color usually exhibited by the major hemolymph lipoproteins has aided in their purification and results from their carotenoid content,

primarily β-carotene and lutein (36, 41), present in a non-covalently bound form (25). Presumably these lipophilic molecules are sequestered in the hydrophobic regions of the lipoprotein particles. In this context, it is highly relevant that we know little of the molecular organization of invertebrate lipoproteins. While the HDL and VHDL of *P. cynthia* appear globular by negative stain electron microscopy, (with mean diameters of 135 and 100 Å, respectively), and exhibit subunit structure (36), it remains largely a matter of conjecture as to whether the insect lipoprotein particles are arranged in a similar overall manner to that suggested in man (52), i.e., with the polar components (proteins and phospholipids) constituting an outer hydrophilic shell, and the apolar constituents (principally neutral lipids, sterol esters (and hydrocarbons ?)) occupying a more hydrophobic core. Some evidence is, in fact, available to indicate that radical differences exist between the structural organization of insect and human lipoproteins. Thus, *M. sexta* HDL, although displaying a similar protein: lipid ratio to the human fraction (Table 1), is larger in diameter (122 Å as compared to 75–95 Å for human HDL) (39, 4), and thus in volume (9.5×10^5 A^3 and 4.5×10^5 A^3 for *M. sexta* HDL and human HDL_3, respectively). Moreover, proteolytic digestion of Manduca HDL and VHDL, in addition to structural and space-filling requirements, indicates an asymmetric arrangement of protein in each particle, with the smaller subunit occupying the hydrophobic core along with the apolar lipids and being essentially immune to tryptic hydrolysis (39, 40). On the other hand, the larger polypeptide in each particle (AL_1 or AV_1) is susceptible to cleavage and largely surface-located; diglycerides, cholesterol, and phospholipids occupy the remainder of the surface. It is tempting to postulate that such a structure may be a direct result of the need for a highly efficient system of lipid transport in this Sphingid.

Finally, the insect hemolymph lipoproteins appear to share many functions with those of the crustacea. Thus, in addition to supplying lipids for metabolic needs, these compounds play fundamental roles in vitellogenesis in both of these arthropod classes and in transporting carotenoids, lipids (as diglycerides), and proteins to the developing ovary. The transport of sterols is also important, since neither insects (53) nor crustacea (54) may synthesize these substances from simple precursors. Recent studies have emphasized the role of hemolymph HDL in the specific binding and transport of juvenile hormone in a number of insect species, including *Locusta migratoria* (55), *Hyalophora gloveri, H. cecropia* and *Antheraea polyphemus* moths (56), *Drosophila hydei* (32) and *Leptinotarsa decemlineata*, the Colorado potato beetle (31), an observation suggesting that hemolymph lipoproteins may be important factors in controlling insect growth.

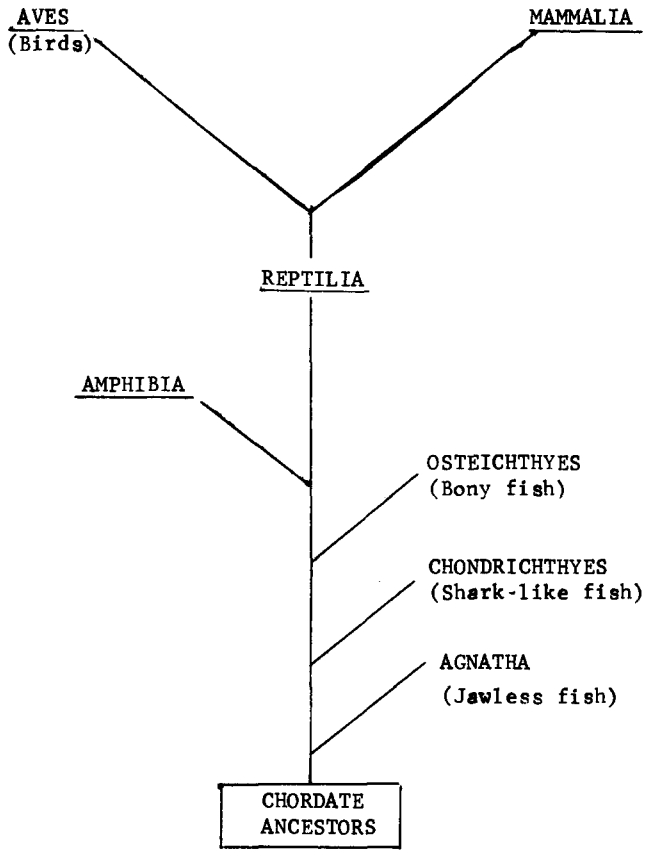

Fig. 2. A simplified family tree showing the evolution and relationship of the main classes of vertebrates.

VERTEBRATE LIPOPROTEINS

Amongst the smallest phyla in the animal kingdom, the vertebrates comprise only about 70,000 species. Nonetheless, as a group they exhibit the greatest evolutionary divergence of all animal species, having developed continuously since the appearance of the earliest recognized representatives, the Cyclostomes, in Ordovician times some 500 million years ago. The vertebrates are generally divided into seven major classes on a taxonomic basis, as indicated in **Fig. 2.** With the exception of the chordate ancestors, studies on the circulating lipoproteins in at least one member species of each of these classes have been described. For present purposes, classes will be subdivided into subclasses, orders and suborders, and when appropriate, into family and genus.

TABLE 4. The concentrations of serum lipoproteins in Cyclostomes and fish[ab]

	VLDL		LDL			
	S_f 100–400	S_f 20–100	S_f 12–20	S_f 0–12	HDL	Reference
Cyclostomata						
Myxine glutinosa (Hagfish)	35–593	515–1074	139–221	189–489	553–753	6,9,57
Elasmobranchii						
Centrophorus squamosus (Shark)	159	256	45	185	40	9,57
Centrophorus granulosus (Shark)	133	132	36	98	23	9
Centroscymnus coelolepsis (Shark)	3	193	93	52	38	9
Scyliorhinus canicula (Dogfish)	0	28	18	136	23	57
Conger vulgaris (Conger eel)	228	228	36	189	N.D.	6,57
Squalus acanthias[c] (Spiny dogfish)	307		120		33	64
Actinopterygii						
Chondrostei						
Acipenser stellatus (Sturgeon)	201	147	9	14	204	9
Acipenser guldenstadtii (Sturgeon)	334	162	76	88	672	9
Huso huso (Sturgeon)	500	226	155	355	649	9
Teleostii						
Pleuronectes platessa (Plaice)	0	9	4	29	N.D.	6
Oncorhynchus nerka[d] (Sockeye salmon)	167		246		238	59
Oncorhynchus gorbuscha (Pink salmon)	0	0	0	0	3300	58
Salmo gairdnerii (Rainbow trout)	0–1	8–46	11–44	105–220	1500	60
Salmo gairdnerii R.[d] (Rainbow trout)	201–212		193–392		1062–2216	61
Sardinops caerulea G.[d] (Pacific sardine)	~115		145		1120	63
Crossopterygii						
Latimeria chalumnae (Coelocanth)	230	875	120	74	127	57,62
Man (urban)[e]	49	83	53	321	230	65

[a] Concentrations are expressed as mg lipoprotein/100 ml of serum.
[b] Distributions were normally determined by analytical ultracentrifugation (57) on pools of serum; S_f is defined as the sedimentation flotation rate in Svedberg units, at a solvent density of 1.063 g/ml at 26°C. HDL is 1.063–1.21 g/ml.
[c] VLDL is of $d < 1.019$ g/ml and LDL, 1.019–1.063 g/ml; values include free fatty acid content and cholesterol as total sterol.
[d] Data based on lipoprotein recoveries after centrifugal flotation; values in *S. caerulea* G. were calculated assuming protein contents of 10% in VLDL, 20% in LDL, and 50% in HDL (63).
[e] Data from fasting males, age 16–29, and excluding HDL_1.
N.D., not determined.

Fishes

As is evident from Fig. 2, there are three major classes of living fishes. These are the Agnates or Cyclostomes, as represented by the hagfish and lamprey, the Chondrichthyes or Elasmobranchs, as represented by the sharks, dogfishes, skates and rays, and the Osteochthyes or bony fish. The latter class is subdivided into two subclasses, i.e., the Crossopterygii, consisting of the lung fishes and coelocanths, and the Actinopterygii, comprising the orders Chondrostei (e.g., sturgeons) and Teleostii. The teleosts are by far the most important of these groups and include the vast majority of modern bony fish.

Typical concentrations of the major classes of serum lipoproteins in a cyclostome, the hagfish *Myxine glutinosa*, and in Elasmobranch, Actinopterygian, and Crossopterygian fishes are compared to those of urban man in **Table 4.** Two points are of particular relevance to these data. First, in cases in which analytical ultracentrifugation has been employed, it has been necessary to define each lipoprotein class by the conventional limits originally proposed by DeLalla and Gofman (66); such limits correspond to minima in the human lipoprotein profile, and may be utterly inappropriate for analyses of certain animal serum lipoproteins, as will be evident in the case of the trout, for example. Second, values such as the refractive increment and concentration dependence of flotation, essential for lipoprotein quantitation, are only available for the human lipoproteins.

The hagfish, *Myxine glutinosa*, is clearly distinguishable from the other fishes and from man in exhibiting elevated concentrations of both its low (i.e., VLDL and LDL) and high density lipoproteins. These values concur with the high cholesterol level (405 mg/100 ml) found in hagfish plasma (67), and may be related to the need for relatively large quantities of cholesterol as a precursor for synthesis of its bile alcohol, myxinol. This compound appears rather ineffective as a fat emulsifier, thereby necessitating its formation in substantial amounts (67, 68).

The principal lipoprotein class in Myxine was the VLDL, accounting for some 50% of the total lipoprotein of $d < 1.21$ g/ml (6, 9, 57). This characteristic was shared by all of the Elasmobranchs, (i.e., the sharks *Centrophorus squamosus*, *C. granulosus* and

centroscymnus coelolepsis (9, 57), the conger eel, *Conger vulgaris* (6, 57), and the spiny dogfish *Squalus acanthias* (64), with the exception of the dogfish *Scyliorhinus canicula* (57), by the sturgeons *Acipenser stellatus* and *Huso huso* (9), and by the coelocanth, *Latimeria chalumnae* (62). The notable and uniform exceptions were the Teleosts, amongst which the plaice (*Pleuronectes platessa*) (6) and late prespawning salmon (*Oncorhynchus gorbuscha*) (58) displayed negligible VLDL levels. This latter observation is however subject to the reserve that seasonal fluctuations in serum lipid levels have been noted in several species of Teleost, which is probably related to spawning.

As a group, the Elasmobranchs were particularly homogeneous and distinct in displaying extremely low HDL concentrations, less than about 10% of the total lipoproteins. In contrast, HDL was the major component in the sturgeons (*A. guldenstadtii* and *H. huso*; 650–670 mg/100 ml serum) (9), and tended to dominate the profiles of the salmonids (58, 60, 81) and Pacific sardine (63) (1060–3300 and 1120 mg/100 ml serum, respectively). LDL concentrations in the sharks, sturgeons, bony fish, and coelocanth were moderate, being less than those of both hagfish and man. Exceptions to this were the high LDL level in the sturgeon *Huso huso* (510 mg/100 ml of LDL) (9); in contrast, in the sturgeon *A. stellatus* (9), the plaice (6), and salmon (*O. gorbuscha*) (58), LDL was either absent or present in trace amounts.

Interestingly, the presence of chylomicrons has been noted in fish plasma. For example, Lauter, Brown, and Trams (64) found such a fraction to account for 25% of the lipid content of spiny dogfish (*Squalus acanthias*) plasma. Moreover, Mills et al. (57) reported small amounts of chylomicron particles of S_f 400–700, in *C. squamosus* serum. We have made similar observations in sera from young rainbow trout fed ad libitum for several days; electron microscopic examination of a negatively stained fraction of d < 1.006 g/ml revealed the existence of numerous small chylomicrons, 800–2000 Å in diameter.[1] Skinner and Rogie (61) have confirmed these findings in electrophoretic and morphological studies of trout VLDL. Some controversy surrounds the nature of fat absorption and transport in fish however, since the studies of Robinson and Mead (69) indicated that dietary [^{14}C]palmitic acid appears in the circulation of rainbow trout as free fatty acid. Indeed, recent studies of a number of fish species, including cyclostomes, elasmobranchs and teleosts, have shown a wide range in the plasma free fatty acid concentration, suggesting that their role as a transport form of lipid is of varying importance (67). This possibility is quite consistent with the great differences which exist in the activities of individual fish and in their mode and sites of lipid storage (70, 71). Thus it is not unreasonable to anticipate some correlation between the type of lipid stored in fish, either in the liver (as in Elasmobranchs), or skeletal muscle, or both, and the lipid composition of the macromolecules in which it is transported, in this case the serum lipoproteins.

The chemical compositions of each of the major lipoprotein classes from species of Cyclostome, Elasmobranch, Chondrost and Teleost are given in **Table 5.** Although certain peculiarities are evident, it may generally be said that the fish lipoproteins are constituted of the same basic components as those of the higher vertebrates and, notably, of man. However, the lower species, i.e., the cyclostome Myxine, the Elasmobranchs (the sharks, *Centrophorus squamosus*, *C. granulosus*, and *Centroscymnus coelolepsis*) and the Chondrosts (the sturgeons, *Acipenser stellatus*, *A. guldenstadtii*, and *Huso huso*), are distinct in that additional types of lipid, i.e., hydrocarbons and monoalkyldiacylglycerols, or hydrocarbons alone are present. Such substances occur rarely in higher vertebrates, though Skipski et al. (44) detected small amounts of hydrocarbons in exhaustive analyses of human serum lipoproteins. Sargent, Gatten, and McIntosh (8) first noted the presence of such primitive lipids, including alkyldiacylglycerols and hydrocarbons, as well as wax esters, in the sera of four species of shark. Since these neutral lipids do not appear to provide a readily utilizable source of energy, it has been suggested that their presence in large amounts in sharks, (particularly as squalene in the liver (8)), is related to the control of their buoyancy, since they lack a swim bladder and must resort to other means for providing lift (8, 72). The low densities of squalene (0.81 g/ml) and alkyldiacylglycerols (0.91) are critical in this regard.

Some uncertainty concerns the presence of wax esters in shark sera (8, 57), which may be explained by the existence of two classes of cholesteryl esters in their lipoproteins, one of which is particularly rich in oleic acid and was found by Mills et al. (57) to migrate to the same position as wax esters on thin-layer chromatography.

The composition of the VLDL in the various species was extremely variable, possibly as a result of differences in the contribution of various particle sizes to each fraction (73). Thus the ranges seen in the proportions of protein, cholesteryl ester, and triglyceride were 3.1–28.4, 2.5–26.7, and 23.0–64.0%, respectively (Table 5). The content of neutral lipids (65%) recognized as being present in the hydrophobic

[1] Chapman, M. J., and L. Fremont. Unpublished observations.

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

Species (Reference)	VLDL											
	Myxine glutinosa (57) (Hagfish)	*Centrophorus squamosus* (57) (Shark)	*Scyliorhinus canicula* (57) (Dogfish)	*Salmo gairdnerii*[a] (60) (Rainbow trout)		*Latimeria chalumnae* (62) (Coelocanth)						Man (6)
Cholesteryl ester	2.5	21.4	8.3	14.9	26.7	3.1						14.9
Free cholesterol	5.1	6.7	7.4	6.4	11.5	7.0						6.4
Triglyceride	48.2	23.0	43.5	49.9	38.5	64.0						49.9
Monoalkyldiacylglycerol	10.6	8.2										
Hydrocarbon	4.4	18.8										
Phospholipid	17.3	15.3	12.4	18.6	16.1	11.5						18.6
Protein	12.1	3.1	28.4	7.7	7.2	14.4						7.7

Species (Reference)	LDL											
	M. glutinosa (57) (Hagfish)	*C. squamosus* (57) (Shark)	*Centrophorus granulosus* (9) (Shark)	*Centroscymnus coelolepsis* (9) (Shark)	*S. canicula* (57) (Dogfish)	*Acipenser stellatus* (9) (Sturgeon)	*Acipenser guldenstadtii* (9) (Sturgeon)	*Huso huso* (9) (Sturgeon)	*S. gairdnerii*[a] (60) (61) (Trout)		*L. chalumnae* (62) (Coelocanth)	Man (6)
Cholesteryl ester	3.0	25.9	20.8	11.5	22.8	13.5	28.7	30.1	15.6	27.9	3.1	38.0
Free cholesterol	9.6	8.4	8.1	7.2	12.5	3.1	3.8	2.5	6.7	9.5	5.8	9.0
Triglyceride	29.5	8.7	5.4	8.6	20.1	30.4	20.6	22.1	26.9	12.5	49.7	11.2
Monoalkyldiacylglycerol	8.7	10.9	12.1	23.9								
Hydrocarbon	5.7	5.9	10.7	5.3		16.9	1.9	6.7				
Phospholipid	22.4	20.5	20.8	22.0	14.8	15.2	22.5	18.9	27.1	14.9	13.6	22.1
Protein	21.1	17.6	22.2	21.5	29.8	21.0	22.5	19.8	24.7	35.2	27.9	20.9

Species (Reference)	HDL								
	M. glutinosa (57) (Hagfish)	*C. squamosus* (57) (Shark)	*S. canicula* (57) (Dogfish)	*S. gairdnerii*[a] (60) (61) (Trout)		*Oncorhynchus gorbuscha*[b] (58) (Salmon)	*L. chalumnae* (62) (Coelocanth)	Man (6)
Cholesteryl ester	0.7	13.9	15.6	7.7	20.1	18.3	1.3	15.0
Free cholesterol	9.6	3.8	9.4	3.4	4.1	3.2	2.2	2.9
Triglyceride	10.5	3.4	12.2	15.5	5.7	6.8	12.5	8.0
Monoalkyldiacylglycerol	1.5	8.0						
Hydrocarbon	6.1	6.9						
Phospholipid	29.6	14.5	9.8	26.5	27.9	30.0	7.0	22.7
Protein	42.1	47.7	53.1	46.9	42.2	39.9	77.1	51.9

[a] VLDL isolated at d < 1.020 g/ml, LDL 1.020–1.085 and HDL 1.096–1.21 g/ml.
[b] Protein content includes 2.4% carbohydrate; free fatty acid content of 1.7%.

core of VLDL in man, (i.e., triglycerides and cholesteryl esters (73, 74)), closely resembled that in the majority of fish species (range 52–67%), with the exception of Myxine and *C. squamosus*. In the VLDL of these animals, the total content of neutral lipids was lower (51% in hagfish and 44% in the shark). In all likelihood, the contribution of cholesteryl ester and triglyceride is diminished in order to accommodate the hydrocarbons and monoalkyldiacylglycerols. Inclusion of these components suggests that hagfish and shark VLDL have core lipid contents of 66 and 71%, respectively, values resembling those in the other fish and in man, and suggestive of a common overall molecular organization in this class of lipoprotein particle.

Further arguments in favor of such a hypothesis derive from the similar morphology of VLDL from the human, Myxine,[2] *C. squamosus* (57), and rainbow trout (60, 61) and from the relationship found between observed VLDL particle diameter and the sum of the content (volume %) of the surface constituents (i.e., protein, phospholipid, and cholesterol) (73). As predicted by the curve (Fig. 9, ref. 73), in trout VLDL, with a mean particle diameter of about 290 Å, about 40% of the content was surface components (60). Similarly, hagfish VLDL, whose surface components amounted to some 35%, had a mean diameter of 310 Å (**Fig. 3**(a) and (b)). These findings

[2] Chapman, M. J., and G. L. Mills. Unpublished data.

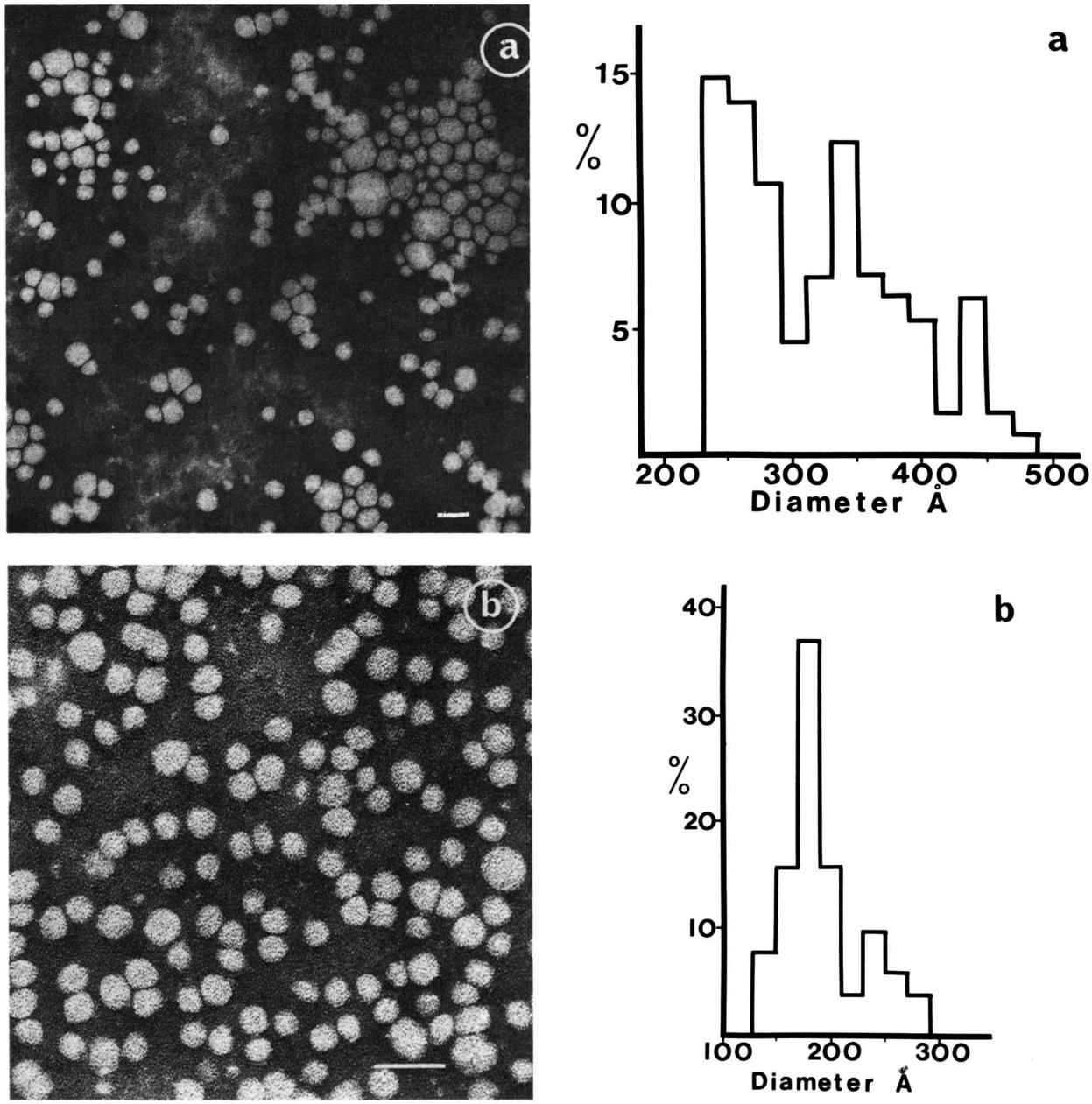

Fig. 3. Particle characteristics and diameters in representative samples of hagfish VLDL and LDL. At left are shown electron photomicrographs of lipoproteins negatively stained with 2% phosphotungstate; each bar represents 500 Å; at right are shown the frequency distributions of lipoprotein particles. The distributions exclude up to 5% of the occasional particles detected at either extreme of the ranges. Fractions are a) Hagfish VLDL (d < 1.006 g/ml) and b) Hagfish LDL (d 1.006–1.063 g/ml).

clearly suggest that hydrocarbons and monoalkyldiacylglycerols sequester into the core of VLDL in hagfish; these particles can thus be envisaged as an oil droplet surrounded by a monomolecular film of constant thickness composed of protein, phospholipids, and free cholesterol (73). The VLDL of both hagfish and coelocanth were distinct in their low proportions of cholesteryl ester (2.5–3.1%), and indeed the ratio of esterified to free sterol in these species (0.2–0.3) was substantially lower than that in man, the elasmobranchs, and trout (0.6–2).

The LDL of the most primitive marine vertebrates examined until now, i.e., the hagfish and the sharks, uniformly contained both hydrocarbons and monoalkyldiacylglycerols. Only hydrocarbons were identified in the sturgeons however, indicating that the monoalkyldiacylglycerols had perhaps become redundant in the course of evolution from these

elasmobranchs to the chondrosts. Such dissimilarities are typical of the diversity seen in the chemical compositions of LDL in the various species (Table 5). Only the phospholipid and protein contents could be said to be relatively consistent and to resemble those characteristic of human LDL. The proportion of free cholesterol was also somewhat invariant, although the sturgeons were distinct as a group in displaying trace amounts (<3.8%).

Marked differences in the relative amounts of triglyceride are evident; while shark LDL was deficient in this lipid, it was the predominant constituent of coelocanth and hagfish LDL. This latter finding is almost certainly related to the major contribution of triglyceride-rich S_f 12–20 substances (equivalent to IDL) to their LDL fractions (Table 4).

Significantly, summation of the lipid components known to distribute into the hydrophobic core of LDL (i.e., cholesteryl ester and triglyceride) (75), together with other neutral lipids, if present, reveals a mean of 49.3% and range of 40.4% (trout) to 60.8% (*A. stellatus*), as compared to 49.2% in human LDL. Such data may be interpreted as indicating that the three-dimensional molecular arrangement in the fish LDL particles may not be significantly different from that of man, i.e., a predominantly hydrophobic core surrounded by the more polar constituents—protein, phospholipid, and free cholesterol (57, 75), even though their individual (neutral) lipid compositions differ considerably. This proposal is entirely consistent with the correlation found between cholesterol and triglyceride content in fish LDL (6). Furthermore, the morphology and mean particle size of LDL in hagfish, *Centrophorus squamosus* and *Salmo gairdnerii*, resemble those of the human fraction. Thus, LDL in hagfish (Fig. 3 (c) and (d)) exhibits a mean diameter of 191 Å, in *C. squamosus*, of 238 Å (57), in *Salmo gairdnerii*, of 157 Å (60), and in man, about 220 Å (57).

A word of caution is in order when considering the chemical compositions of LDL fractions, not only in fish but also in higher vertebrates. To the author's knowledge, only in the case of the rainbow trout (60, 61) has the density distribution of a fish LDL been precisely evaluated with sensitive analytical techniques directed primarily to identification of its principal apolipoprotein; in trout, LDL was distributed up to densities of 1.078–1.085 g/ml. Any study in which quantitation and characterization of LDL are performed employing a fraction of d 1.006–1.063 g/ml would therefore be misleading. Furthermore, information such as density distribution may provide valuable insight into LDL metabolism. Some reevaluation of the data discussed above may be necessary should the LDL of any of these fish be distributed differently from that of man to a significant degree. Such differences may well escape detection by the analytical centrifuge, since material in the region of the 1.063 g/ml boundary cannot be identified as low-density (apo-B-containing) or high-density (apo-A-containing) unless specific antisera or gel electrophoretic techniques are employed for apoprotein analysis of narrow density fractions prepared with high resolution fractionation procedures (such as density gradient ultracentrifugation (60)).

There were deficiencies in cholesteryl ester of the VLDL and LDL of Myxine and Latimeria as well as in HDL, presumably indicating a lack of cholesterol-esterifying (LCAT) activity and a differing role for this lipid in these species. The HDL of Myxine also contained the hydrocarbon and monoalkyldiacylglycerol components typical of their VLDL and LDL, as did the shark (*C. squamosus*) HDL. The compositions of HDL tended to show wide variations between the Cyclostomes, Elasmobranchs, Teleosts and man, and only that of the Teleosts (pink salmon and rainbow trout) resembled the human HDL to any extent. Such variability may provide an informative means by which to probe the role of individual components in HDL structure, even though certain of the physical properties of rainbow trout and pink salmon HDL (58, 60) resemble those of man more closely than might be anticipated on the basis of their chemical compositions. Thus, by electron microscopy, trout HDL range in diameter from 60–110 Å and exhibit a mean diameter of 77 Å (60), while the Stokes radius of the salmon fraction is 80 Å and its molecular weight 180,000 (58), values indicating a close correspondence to human HDL_3 (4).

A denser form of HDL, i.e., a very high-density lipoprotein, has also been identified in fish serum (76–78). This substance is implicated in egg formation and apparently corresponds to a vitellogenin (76), having been isolated from both female trout (*S. gairdnerii* (76, 78)) and cod (*Gadus morhua* L. (77)) sera, and found to correspond in size (76, 77), in immunological reactivity (76, 78), and in protein content (77) to the egg lipoprotein itself. The trout serum VHDL (d > 1.21 g/ml) possessed a similar composition to the egg lipoprotein extracted from the eggs, with lipid and protein contents of 23 and 77%, respectively (76); their lipid components broadly resembled those of the serum HDL. Both trout and cod sera VHDL appear to exist in two forms, although this may be the result of the precipitation procedure employed in their isolation (76, 77). None of the serum apolipoproteins could be detected in the serum VHDL of either trout or cod, indicating that certain specific serum proteins are selectively involved in fish vitello-

TABLE 6. Phospholipid distribution in fish lipoproteins

Species (Reference)	VLDL		LDL							HDL		
	M. glutinosa (79) (Hagfish)	*S. gairdnerii* (79) (Trout)	*M. glutinosa* (9) (Hagfish)	*C. squamosus* (9) (Shark)	*C. granulosus* (9) (Shark)	*C. coelolepsis* (9) (Shark)	*A. guldenstadtii* (9) (Sturgeon)	*H. huso* (9) (Sturgeon)	*S. gairdnerii* (79) (Trout)	*M. glutinosa* (9) (Hagfish)	*S. gairdnerii* (79) (Trout)	*O. gorbuscha* (58) (Salmon)
	% (by weight)											
Phosphatidylcholine	73.4	81.3	61.4	63.5	54.6	72.3	74.5	71.2	74.2	63.3	84.0	83.1
Sphingomyelin	6.3	11.7	12.9	28.0	23.8	20.8	5.6	7.5	11.6	15.6	7.8	6.7
Phosphatidylethanolamine	11.9	2.6	7.4		1.3	4.7	5.6	2.9	4.0	10.3	1.4	2.3
Phosphatidylserine	2.0	1.5	2.8		1.3		1.9	2.9	3.7	3.1	1.1	3.9
Phosphatidylinositol												
Lysolecithin	4.3	2.9	12.0	6.9	17.2	2.1	9.9	11.7	4.5	5.3	4.2	3.1

genesis, at least in Teleosts (76, 77). This situation is clearly distinct from that found in the invertebrates in which the serum apolipoproteins, present in both high density and very high density lipoproteins, perform the dual role of transporting lipids for metabolic requirements as well as other components (e.g., proteins, lipids, and carotenoids) for egg formation. Of the lipid components of fish lipoproteins, the phospholipids are rather unremarkable (**Table 6**), their distribution differing little from that found in man (Table 2). Thus, phosphatidylcholine is found as the predominant species; only the high lysophosphatidylcholine content of Myxine, *C. squamosus*, *A. guldenstadtii*, and *H. huso* LDL is noteworthy. The elevated phosphatidylethanolamine content of all three classes of hagfish lipoproteins tends to distinguish them, though this is not as high as that seen in the insect compounds (Table 2).

The fatty acids of the lipid esters of fish lipoproteins are a most characteristic feature of these vertebrates, and represent a specific adaptation to their life as poikilotherms or non-heat sustaining animals. Thus, the need to maintain their lipids in a liquid phase at low temperature is manifested in elevated proportions of highly unsaturated long chain fatty acids. For example, the degree of unsaturation may reach 95% or more in exceptional instances, such as is the case in cholesteryl ester fraction 2 (CE2) in the VLDL, LDL, and HDL of *C. squamosus* (57) and of *S. gairdnerii* (60) (**Table 7**); some 70% of the fatty acids in the former are constituted by the 20- and 22-carbon penta- and hexaenoic forms, 20:5 and 22:6, while some 65% of those in the latter consist of the 22:6 form alone. These observations imply a rather specific role for the CE2 fraction in the transport of the essential fatty acids, i.e., eicosapentaenoate and docosahexanoate, in the shark and trout. Enormous complexity also exists in the constitutive fatty acids of fish lipoproteins, a remark best illustrated by the nearly 90 different acids detected by Nelson and Shore (58) in the lipid esters of salmon HDL.

Although the need for highly unsaturated lipids is uniform in fish, some variation is evident in the fatty acid composition of the lipid esters in different fish (Table 7). However, an overall similarity exists in the fatty acid pattern of each lipid ester in the major lipoprotein classes of any given species. Thus the cholesteryl esters of the VLDL, LDL, and HDL of *Myxine*, *C. squamosus* (i.e., CE 1), and Latimeria show an overall resemblance, being rich in the 16:0, 16:1, and 18:1 acids (57, 62). In contrast, the cholesteryl esters of the LDL and HDL of the Pacific sardine (63) are relatively poor in palmitoleate (especially LDL) and oleate; they exhibit elevated proportions of the penta- and hexaenoic acids 20:5 and 22:6, thereby resembling the shark lipoprotein CE 2 fraction. Similarly, while the triglycerides of *S. caerulea* (63) displayed high contents of the 16:0, 18:1, 20:5, and 22:6 acids, those of Latimeria were rich in 16:0, 16:1, 18:1, and 18:3 (57), and those of *S. gairdnerii*, in 16:0, 16:1, 18:1, 18:2, and 22:6 (55); in the trout and coelocanth, oleic acid was the major component. In the phospholipids, the 16:0, 18:0, and 18:1 forms predominated in the coelocanth; 16:0, 18:0, 18:1, 18:2, and 22:6 in the trout, and 16:0 (HDL), 16:1 (LDL), 18:1, 20:5, and 22:6 acids in the sardine; oleate was the major phospholipid fatty acid in the lipoproteins of the two former fish while docosahexaenoic acid tended to predominate in the sardine lipoproteins. The fatty acid patterns of fish lipoprotein lipids thus serve to distinguish these marine vertebrates from their terrestrial counterparts, not only in their high degree of unsaturation, but also in their apparent deficiency of arachidonic acid (20:4), so common to higher animals.

TABLE 7. Fatty acid compositions of the lipid esters of fish lipoproteins

Species	(Ref.)	Lipoprotein Fraction	Lipid	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:2	20:4	20:5	22:0	22:1	22:4	22:5	22:6	Uniden.
													%								
Latimeria chalumnae (coelocanth)	(62)	VLDL	CE	2.0		17.0	11.5	6.2	57.6	1.7	2.8			1.6							
Centrophorus squamosus (shark)	(57)	VLDL	CE (1)	2.8		11.4	8.8	2.8	62.9	2.6		5.6		1.4							1.8
Centrophorus squamosus (shark)	(57)	VLDL	CE (2)	0.2		2.0	0.7	0.9	1.9	0.6	0.6			9.7	37.5				8.6	33.4	4.0
Salmo gairdnerii (trout)	(60)	VLDL	CE (1)	0.6		25.7	4.3	6.5	28.5	9.4	12.7		5.9	4.1							1.0
Salmo gairdnerii (trout)	(60)	VLDL	CE (2)	0.8		7.0	2.4	3.1	3.3	0.8				5.5	6.7					65.5	5.0
Latimeria chalumnae (coelocanth)	(62)	LDL	CE	3.2		19.1	12.7	7.5	46.0	2.5	3.6			5.5							
Centrophorus squamosus (shark)	(57)	LDL	CE (1)	2.2		12.1	8.0	3.3	58.2	3.6		6.3		3.7							2.6
Centrophorus squamosus (shark)	(57)	LDL	CE (2)	0.3		1.9	0.6	1.1	1.1	0.6	0.7			11.9	38.1			1.2	5.4	32.8	4.3
Myxine glutinosa (hagfish)	(62)	LDL	CE			6.1	3.7	5.8	29.6	3.0	21.5			30.3							
Scyllium canicula (dogfish)	(62)	LDL	CE			2.8	3.8		12.7	1.9	0.4			15.7	21.4				0.9		38.8
Salmo gairdnerii (trout)	(60)	LDL	CE (1)	0.6		30.5	4.0	8.5	25.2	8.2	11.4		5.3	4.0		1.3					1.2
Salmo gairdnerii (trout)	(60)	LDL	CE (2)	0.7		4.8	2.3	2.5	2.5	1.3	0.8		1.0	5.9	6.9	1.2				64.5	5.8
Sardinops caerulea G. (sardine)	(63)	LDL	CE	1.3		15.9	0.9	1.2	6.5	0.3		0.1		0.8	19.7					52.1	
Latimeria chalumnae (coelocanth)	(62)	HDL	CE	1.4		20.7	8.8	15.8	40.2	2.4	6.7			4.2							
Centrophorus squamosus (shark)	(57)	HDL	CE (1)	2.0		14.6	7.5	3.9	55.3	3.5		5.7					5.2				2.4
Centrophorus squamosus (shark)	(57)	HDL	CE (2)	0.3		2.9	1.0	1.6	1.4	0.6	0.6			11.8	36.2			1.8	4.8	32.8	4.0
Salmo gairdnerii (trout)	(60)	HDL	CE (1)			28.7	4.3	7.5	30.0	9.6	12.8		4.9	2.3							
Salmo gairdnerii (trout)	(60)	HDL	CE (2)	0.5		4.2	2.1	1.8	2.8	3.2	0.5			6.0	6.5	2.0			66.9		
Sardinops caerulea G. (sardine)	(63)	HDL	CE	3.3	0.3	22.7	6.8	1.6	12.0	0.6		0.3	0.5	0.3	15.5				0.2	35.1	
Latimeria chalumnae (coelocanth)	(62)	VLDL	TG	2.6		14.5	10.1	1.3	62.1	0.5	6.6			1.7							
Centrophorus squamosus (shark)	(57)	VLDL	TG	2.0		18.9	6.8	3.5	39.5	0.8		5.8			4.3		4.5			12.4	1.5
Salmo gairdnerii (trout)	(60)	VLDL	TG	1.8		16.6	9.0	3.5	41.9	10.1	5.8		2.7	1.9						6.9	
Latimeria chalumnae (coelocanth)	(62)	LDL	TG	1.0		14.4	8.3	3.2	59.2	1.1	8.5			4.3							
Centrophorus squamosus (shark)	(57)	LDL	TG	1.5		17.8	6.0	3.8	32.8	1.2		5.7		1.7	4.8		4.5			17.3	3.1
Myxine glutinosa (hagfish)	(62)	LDL	TG	1.0		14.4	8.3	3.2	59.2	1.1	8.5			4.3							
Scyliorhinus canicula (dogfish)	(62)	LDL	TG			8.8	3.7	3.4	19.2	0.9	2.3			3.1	20.5				1.0		35.3
Salmo gairdnerii (trout)	(60)	LDL	TG	1.5		17.2	8.9	3.6	42.8	10.4	5.2		1.6	0.7						8.2	
Sardinops caerulea G. (sardine)	(63)	LDL	TG	3.9	0.6	24.7	7.9	2.1	17.0	1.5		1.1	3.2	3.3	16.4			0.2	1.9	10.9	
Latimeria chalumnae (coelocanth)	(62)	HDL	TG	1.8		14.4	11.7	5.4	44.2	1.4	8.5			1.5							
Centrophorus squamosus (shark)	(57)	HDL	TG	1.5		17.8	6.0	3.8	32.8	1.2		5.7		1.7	4.8		4.5			17.3	3.1
Salmo gairdnerii (trout)	(60)	HDL	TG	1.6		17.2	8.9	3.5	40.7	10.3	4.8		2.0	1.4						9.7	
Sardinops caerulea G. (sardine)	(63)	HDL	TG	5.6	0.7	24.2	7.4	3.8	15.7	1.2		1.5	3.7	2.5	20.5	0.4			1.9	9.5	
Centrophorus squamosus (shark)	(57)	VLDL	MAGE	1.7		20.7	4.9	3.4	36.2	3.6		7.5					8.2			11.4	2.5
Centrophorus squamosus (shark)	(57)	LDL	MAGE	1.6		16.2	3.7	3.3	26.7	2.0		8.7			2.5		11.9		2.3	14.2	7.0

Centrophorus squamosus (shark)	(57)	HDL	MAGE	1.8	18.6	4.4	5.9	27.0	10.1	1.2	6.6		1.9		7.6		11.8	3.1
Latimeria chalumnae (coelocanth)	(62)	VLDL	PL	2.3	20.6	8.2	13.6	49.0	2.7	9.2		15.1						
Centrophorus squamosus (shark)	(57)	VLDL	PL	1.0	42.6	3.7	2.8	23.4	1.7		0.8		4.7	7.1			11.0	1.2
Salmo gairdnerii (trout)	(60)	VLDL	PL	1.2	25.0	3.5	11.5	14.1	8.3	2.3		2.1	1.6		1.8		23.7	
Latimeria chalumnae (coelocanth)	(62)	LDL	PL	2.1	18.1	8.1	11.3	41.0	2.9	5.4			11.1					
Centrophorus squamosus (shark)	(57)	LDL	PL	1.9	35.6	3.6	3.6	19.0	1.9		1.4		4.5	7.5			18.5	2.6
Myxine glutinosa (hagfish)	(62)	LDL	PL		7.7	4.6	9.0	35.7	1.7	6.5		8.1		7.0		14.2		
Scyliorhinus canicula (dogfish)	(62)	LDL	PL		19.7	2.3	2.9	11.7	0.1	2.0								42.5
Salmo gairdnerii (trout)	(60)	LDL	PL	1.2	25.9	3.3	11.6	14.6	8.7	2.5							25.6	
Sardinops caerulea G. (sardine)	(63)	LDL	PL	1.6	0.2	1.3	35.0	3.3	7.7	0.7	0.2	0.4		1.8	11.8		0.6	0.6	25.5	
Latimeria chalumnae (coelocanth)	(62)	HDL	PL	0.4		11.3	4.5	11.7	42.1	2.6	0.9			1.8						
Centrophorus squamosus (shark)	(57)	HDL	PL	1.3		32.1	3.4	4.9	21.0	1.4		1.3		5.4	7.2		3.0		16.6	2.3
Salmo gairdnerii (trout)	(60)	HDL	PL	1.0		24.8	2.9	10.5	13.3	8.7	3.0		2.3	2.4		2.7			28.6	
Sardinops caerulea G. (sardine)	(63)	HDL	PL	0.8		28.6	3.5	0.3	6.1	0.6			0.7	4.5	17.3				37.4	

Values are weight % of fatty acids recovered.
CE, cholesteryl ester; TG, triglyceride; MAGE, monoalkyldiacylglycerol; PL, phospholipid; CE (1) and CE (2) represent two forms of CE separated by thin-layer chromatography (57).
Uniden., unidentified fatty acids.
Fatty acids present in trace amounts (<0.1%) omitted.
Contents of the 14:1, 15:1, 16:2, 17:0, 17:1, 19:0, 20:0 fatty acids in sardine lipid esters (63) less than 1%; the 14:1 represented 8.9% of LDL phospholipid fatty acids and the 22:2 form, 2.8% of LDL triglyceride fatty acids.

Indeed, in only the fatty acid pattern of coelocanth lipoproteins has any degree of similarity with the mammalian compounds been found (62).

By way of contrast to the fatty acids, the alkyl moieties of the glyceryl ethers of Myxine and the sharks (*Centrophorus squamosus*, *C. granulosus*, and *Centroscymnus coelolepsis*) are principally 16 and 18 carbon atoms in chain length and lack a high degree of unsaturation (9, 57). The major species was the 18:1 compound, corresponding to the selachyl alcohol found upon hydrolysis of the monoalkyldiacylglycerol (57).

Investigations of the protein moieties of fish serum lipoproteins have been described in only a few species, i.e., in the Cyclostome Myxine (9, 80), in the Elasmobranch *Centrophorus squamosus* (57), and in three Teleosts, the salmonids *Salmo gairdnerii* (60, 61) and *Oncorhynchus gorbuscha* (58), and the cod (*Gadus morhua* L.) (81); observations in both *O. gorbuscha* and *G. morhua* are restricted to the HDL class.

The nature of the apoproteins of fish VLDL and LDL on the one hand, and of their HDL on the other, has been examined by immunological and chemical analyses (57, 60, 61). These have shown that the low (i.e., VLDL and LDL) and high density lipoproteins possess distinct antigenic determinants and amino acid compositions in the hagfish,[2] the shark *Centrophorus squamosus* (amino acid profile (57)), and the rainbow trout (60, 61). This situation clearly resembles that in man (7), in which the B apolipoprotein is the major component of both VLDL and LDL, while the A apolipoproteins predominate in HDL.

The presence in fish low density lipoproteins of a counterpart to the human B protein was first suggested by the solubility behavior of the delipidated protein moieties, inasmuch as they were incompletely soluble in aqueous buffers in the presence of high urea concentrations. They were entirely soluble, however, in solutions containing amphipathic detergents, such as dodecyl and decyl sodium sulfates. This suggestion proved valid when the detergent-solubilized protein moieties were electrophoresed in SDS-polyacrylamide gel, in which case the major component(s) was consistently resolved as an intense diffuse band of high molecular weight (250,000 or greater) (57, 60); such behavior is typical of human apo-B (82, 83). These findings led to the fractionation of the apoproteins of hagfish (80) and shark LDL (57), and of trout VLDL and LDL-2 (d 1.024–1.078 g/ml) (60) by gel chromatographic techniques based on those originally used for separation of the B and C proteins from human apo-VLDL by Brown, Levy, and Fredrickson (84) and by Herbert et al. (85). The apolipoprotein B-like components isolated in this way accounted for about 35% of the total apoprotein of trout VLDL and

TABLE 8. Amino acid compositions of fish apolipoproteins resembling the human B and A-I proteins

Species	*Myxine glutinosa* (Hagfish)	*Centrophorus squamosus* (Shark)	*Salmo gairdnerii* (Trout)		Man	*S. gairdnerii* (Trout)	*Oncorhynchus gorbuscha* (Salmon)		Man
Parent lipoprotein	LDL	LDL	VLDL	LDL-2[a]	LDL	HDL	HDL		HDL
Apolipoprotein	Apo-B	Apo-B	Apo-B		Apo-B	A-I	A-I[b]		A-I[c]
(Reference)	(80)	(54)	(60)		(86)	(61)	(58)		(3)
Amino acid							(3)	(4)	
Lysine	6.8	9.2	5.7	7.3	8.0	9.9	9.7	9.9	8.7
Histidine	2.0	2.4	1.6	1.2	2.2	1.2	1.6	1.6	2.1
Arginine	4.0	4.2	2.8	2.8	3.3	6.5	4.7	5.4	6.6
Aspartic acid	13.2	11.5	12.4	11.6	10.8	5.4	5.8	5.5	8.7
Threonine	5.5	7.3	8.0	8.4	6.5	4.3	5.3	5.5	4.2
Serine	9.9	9.5	8.5	6.5	8.6	4.1	4.9	4.8	5.8
Glutamic acid	11.9	11.5	12.6	13.6	12.5	18.1	19.9	20.5	19.5
Proline	4.0	3.0	1.0	0.9	4.0	4.8	3.8	3.8	4.1
Glycine	6.6	7.0	5.5	4.7	5.0	2.6	2.1	2.2	4.1
Alanine	6.9	6.6	9.2	10.8	6.5	13.2	12.0	11.5	7.9
Valine	4.1	5.3	9.4	9.5	5.0	6.7	6.5	8.0	5.4
Methionine	3.0	0.9	2.7	1.4	1.4	2.4	3.3	3.2	1.3
Isoleucine	3.9	5.3	4.9	5.0	5.2	4.5	3.7	3.2	0
Leucine	10.3	9.7	9.2	11.2	12.2	10.3	11.0	10.2	16.2
Tyrosine	3.1	2.8	2.6	2.3	3.3	3.7	4.0	3.8	2.9
Phenylalanine	4.8	3.8	2.8	2.9	5.2	2.3	1.6	1.1	2.5

[a] Trout LDL-2 was of d 1.020–1.078 g/ml (60).
[b] DEAE fractions (3) and (4).
[c] Calculated from the primary structure.

varying proportions (60–90%) of that of hagfish, shark, and trout LDL; their amino acid compositions are presented in **Table 8.** The amino acid profiles are markedly alike and closely resemble that of human apo-B; moreover, the apo-B-like components from trout apo-VLDL and apo-LDL-2 are indistinguishable from each other. However, despite the fact that these proteins appear to be analogous to the human protein, the various fish apo-B's did not cross-react to any appreciable extent with antisera to either human LDL or apo-B in immunological analyses (80), thereby suggesting the existence of fundamental differences in the structure of their antigenic determinants. Nevertheless, the existence in the lowest vertebrates of an apolipoprotein analogous to human apo-B indicates it to be a fundamental, and highly-specialized, lipid-transporting protein.

The major apolipoproteins of HDL in the rainbow trout (60, 61) and pink salmon (58) appear analogous to the A proteins of man. Thus, in these two salmonids, as well as in the cod (81), two proteins predominate; electrophoresis in basic-urea polyacrylamide gel of tetramethylurea- or urea-solubilized apo-HDL resolved two major bands in the trout and salmon fractions which corresponded closely in their migration to the human A-I and A-II proteins (58, 60, 61). The fish and human proteins were also alike in their behavior on gel filtration (61) and ion-exchange chromatography (58); the former finding suggested similar molecular weights for the trout and human polypeptides. This was confirmed by estimation of their size by electrophoresis in SDS-polyacrylamide gel which showed that their molecular weights were in the ranges 10,000–11,400 and 22,000–26,500 (60). These are comparable to the A-II and A-I components of human HDL, whose molecular weights are, respectively, 8,690 (monomer) and 28,300 (3, 4). Comparison of the amino acid compositions of one of the major HDL apolipoproteins from the trout (gel filtration fraction II (61)) and from the salmon (DEAE fractions 3 and 4 (58)) with that of human A-I revealed a close resemblance (Table 8). Minor differences are however apparent, of which the most notable are the presence of isoleucine, the elevated content of alanine, and lesser amounts of aspartic acid and leucine in the fish proteins. The A-I polypeptides of salmon and man are also alike in being polymorphic (4, 58). The second major HDL apolipoprotein in trout has not been characterized to date, but the salmon protein (DEAE fraction 2) differs considerably in amino acid composition from human A-II, especially in its lack of cystine (58). In this respect, it resembles A-II of the rat, dog, rabbit, and rhesus monkey, in which monomeric forms of apo-AII have also been detected (3, 4). Indeed, apart from man, the chimpanzee appears the only species as yet to have a dimeric apo-AII (3, 4). Although little information is available on the wider occurrence of this protein in animals, it appears that some vertebrates, such as the cow (87), may lack apo-AII entirely; in others, monomeric

TABLE 9. Serum lipoprotein concentration in amphibians and reptiles[a,b]

Species	VLDL		LDL		HDL	Ref.
	S_f 100–400	S_f 20–100	S_f 12–20	S_f 0–12		
Amphibians						
Rana temporaria (Frog)	0	99	38	55	29	(6)
Rana catesbeiana (Bullfrog)						
Adult	10–30		55–65		5–10	(88)
Tadpole	N.D.		N.D.		200–300	(88)
Pleurolides waltii (Salamander)	0	2	6	6	17	[c]
Reptiles						
Natrix natrix (Grass snake)	149	58	48	359	382	(6)
Liopeltis vernalis (Green snake)	0	64	92	1168	N.D.	(6)
Natrix piscator (Water snake)	84	213	211	267	222	(6)
Ptyas mucosus (Rat snake)	0	43	111	320	N.D.	(6)
Varanus salvator (Water monitor)	0	0	0	130	58	(6)
Testudo graeca (Tortoise)	0	0	0	45	117	(6)

[a] Concentrations are expressed as mg lipoprotein/100 ml serum.
[b] Distributions determined by analytical ultracentrifugation (57).
[c] Values in the salamander *P. waltii* are from the unpublished data of Chapman, M. J., M. Flavin, and G. L. Mills.
N.D., not determined.

analogues may be present in as yet uncharacterized apo-C fractions.

Further dissimilarities in the apoprotein content of fish and human lipoproteins were seen in the complement of low molecular weight polypeptides, (soluble in tetramethylurea), in the trout lipoproteins (60). Thus, polyacrylamide gel electrophoresis indicated the trout lipoproteins to be deficient in rapidly-migrating components analogous to the C-peptides in man (60). Even so, a counterpart to human apo-C-II may be present in the trout, since its VLDL could act as a substrate for purified bovine milk lipoprotein lipase.[3]

It is abundantly clear from the above discussion that the lipoprotein systems of even the most primitive vertebrates approach those of the mammals and man in their complexity, thereby indicating the early evolutionary appearance of these macromolecules. Our further knowledge of the molecular structure of 'simple' lipoproteins must therefore await study of animals substantially lower on the evolutionary scale than the fish, and, indeed, than the insects.

Amphibians and Reptiles

The class Amphibia is subdivided into three subclasses, represented by the caecilians (Apoda), the newts and salamanders (Urodela) and the frogs and toads (Anura). They appeared during the late Devonian period and are closely related to the reptiles, which arose somewhat later during carboniferous times.

The principal subclasses of living reptiles are the Anapsids and Diapsids; the major order in the former is that of the Chelonia (turtles and tortoises) and the principal orders of the latter are the Squamata (lizards and snakes) and Crocodilia (alligators and crocodiles). Since the amphibians and certain of the reptiles have retained the aquatic habitat of the fish, while others have evolved to adapt to a terrestrial environment, one might expect the study of their lipoproteins to reflect any evolutionary changes necessary in lipid transport for a successful life on land. Unfortunately however, the amphibians and reptiles are the most poorly studied of the vertebrates and only superficial knowledge of their serum lipoproteins is presently available. This extends to analyses of serum lipid and lipoprotein levels, electrophoretic profiles and rather meager chemical data.

The serum lipoprotein profiles of a number of amphibians and reptiles are summarized in **Table 9.** Lipoprotein levels appear rather low in the amphibians, in which only trace amounts of high density substances were identified. The exception to this was the bullfrog tadpole, which has been reported (88) to possess HDL levels of the same order as those of man. Similar concentrations of HDL were also seen in the snakes. These reptiles were readily distinguished from the amphibians however, since they displayed elevated concentrations of low density lipoproteins of S_f 0–20, and also occasionally of VLDL. In fact, with the exception of the water monitor, the snake levels of S_f 0–12 LDL were so high (range 407–1260 mg/100 ml) as to exceed those of man (~350 mg/100 ml (6, 65)), making them almost unique in the animal kingdom.

The tortoise differed somewhat from the other reptiles, in that although it possessed a moderate quan-

[3] Glangeaud, M. C. Personal communication.

tity of HDL, it lacked VLDL and its LDL concentration resembled that of the amphibians. Additional studies in other species of the same order (i.e., Chelonia) will be needed to determine whether this type of profile is common to them.

A good example of the occasional limitation met when imposing conventional density limits on an animal lipoprotein profile is afforded by the water monitor (*V. salvator*). Analytical ultracentrifugation (at a solvent density of 1.063 g/ml) suggests that this reptile displays an essentially continuous distribution of lipoproteins which is transected by the 1.063 g/ml boundary (6). It seems therefore that in the monitor there may be only a single lipoprotein class, as Mills and Taylaur (6) suggested on the basis of the similarity in composition of the 'LDL' and 'HDL' of this poikilotherm (Table 9). Alternatively, this finding could be explained by the presence in the 'HDL' fraction of a relatively large proportion of LDL particles and a small amount of true high density lipoproteins, a situation typified by the guinea pig, whose apo-B containing, low density lipoproteins are distributed up to a density of 1.100 g/ml and which possesses HDL concentrations of 10 mg/100 ml serum or less (89, 90). While biochemical studies are needed to differentiate between these two possibilities, the relatively low protein and high cholesteryl ester contents (28.9 and 40.4%, respectively) of the total monitor 'HDL' suggest that its predominant lipoprotein species resembles an LDL particle more than a true HDL. The general nature of the lipoprotein profiles in amphibians and reptiles, (i.e., low concentrations of all lipoprotein classes in the amphibians, with relatively high concentrations of LDL and HDL in the reptiles, LDL predominating), has been confirmed in other studies in which less accurate procedures have been employed for quantitation, notably electrophoresis in agarose gel or other media (91, 92).

The chemical compositions of the various amphibian (bullfrog and frog) and reptilian (grass snake, water snake, water monitor, and tortoise) lipoproteins are compared with each other and with the corresponding fractions (VLDL, d < 1.006, LDL d 1.006–1.063, and HDL d 1.063–1.21 g/ml) from human serum in **Table 10.** The VLDL fractions were alike in that their contents of neutral lipid (triglyceride and cholesteryl ester) were within the range 60–65%; similarly, their proportions of polar constituents (phospholipid and protein) varied only from 23.6 to 26.3%. The greatest dissimilarity was in free cholesterol content, which was highest in the VLDL of the water snake (13.0%) and lowest in man (6.7%). This was of course reflected in the ratios of esterified to free cholesterol (EC/UC) which were essentially the same in bullfrog and man

TABLE 10. Mean weight percent chemical composition of amphibian and reptilian serum lipoproteins

Species (Reference)	VLDL				LDL						HDL					
					Bullfrog											
	Bullfrog (88)	Grass Snake (6)	Water Snake (6)	Man (6)	Adult	Tadpole (93)	Frog (6)	Grass Snake (6)	Water Snake (6)	Water Monitor (6)	Tortoise (6)	Man (6)	Bullfrog (88)	Frog (6)	Grass Snake (6)	Water Snake (6)	Water Monitor (6)	Tortoise (6)	Man (6)
Cholesteryl ester	17.3[a]	4.6	9.2	14.9	30.3	37.6	42.5	35.0	33.2	42.4	20.2	38.0	19.3[a]	22.4	24.4	21.8	40.4	14.3	15.0
Free cholesterol	8.8	7.9	13.0	6.7	5.6	3.2	13.9	13.0	14.6	13.5	9.4	9.0	11.9	10.3	7.3	14.8	12.1	3.4	2.9
Triglyceride	48.1	61.7	52.9	49.9	12.5	16.0	1.4	5.8	11.0	5.8	23.9	11.2	3.7	3.1	3.5	4.7	4.5	5.9	8.0
Phospholipid	11.6	14.4	14.4	18.6	23.3	22.4	16.8	17.7	18.2	11.2	16.1	22.1	25.2	13.1	24.7	21.6	14.2	12.9	22.7
Protein	12.0	11.4	10.6	7.7	22.4	20.0	25.4	28.5	23.0	27.1	30.5	20.9	36.8	51.0	40.0	37.1	28.9	63.5	51.9

[a] The free fatty acid and partial glyceride contents (total 2–3%) of bullfrog VLDL and HDL are omitted (88).

(1.3 and 1.2, respectively) but substantially lower in the snakes (0.4).

In a similar way, the esterified/free cholesterol ratios of human and bullfrog (adult and tadpole) LDL's were of the order of twofold or more greater than those of the other amphibians and reptiles. Large variations in cholesteryl ester (20.2% in the tortoise and 42.5% in the frog) and triglyceride (1.4% in frog and 13.9% in tortoise) were seen, although as in VLDL, the total neutral lipid content varied less (40.8–53.6% in grass snake and bullfrog tadpole, respectively). Again, the hydrophilic constituents (protein and phospholipid) accounted for a similar proportion of the total particle weight in amphibians, reptiles, and man, (38.3–46.6%), suggesting that the peak hydrated density (and thus overall particle distribution) of each LDL fraction, even though isolated over a wide density range and continuing a heterogeneous mixture of molecular species, might be rather similar. This was indeed the case, the peak of the major ultracentrifugal fraction (S_f 0–12) showing a narrow range (4.1–7.5 in the grass snake and tortoise, respectively and 7.0 in man (6)). The protein content of human LDL was less than that seen in all the amphibian and reptilian fractions; little emphasis may be placed on this difference, however, since variation in the distribution of LDL within the interval 1.006–1.063 g/ml could readily explain this finding. Future studies of the LDL (and VLDL) of these (and other) species should therefore be performed on fractions isolated within a narrower density interval, e.g., 1.020–1.050 g/ml, or if this is inappropriate, on the major LDL particle species isolated, for example, by density gradient centrifugation.

HDL was characterized in all species by a high protein content, with the exception of the water monitor (see above). Exclusive of *V. salvator*, the proportion of cholesteryl ester showed a relatively narrow range (14.3–24.4%). The wide variation in the ratio of esterified/free cholesterol is explained by large species differences in HDL free cholesterol. The two amphibians tended to display lower esterified/free cholesterol ratios than the other species, bullfrog, frog, and man, 1.0, 1.3, and 3.1, respectively; this trend was not noted in their VLDL or LDL however. While triglyceride varied minimally, phospholipids ranged widely, from 12.9% in the tortoise to 25.2% in bullfrog, resulting in a large variance in the total particle weight of polar components (phospholipid and protein) from 58.7% in *N. piscator* to 76.4% in tortoise. This finding could be interpreted either as suggesting that the nature of the major HDL species was somewhat variable from animal to animal, its lower or higher hydrated density being reflected in the altered proportions of such components, or alternatively, that the structural role of these molecules may not be exactly the same in the HDL of each species. Some evidence for a coat/core relationship in HDL structure in both reptiles and fish has been provided however by the observation that protein and cholesteryl ester content are significantly correlated (6).

The only published data on the phospholipid composition of amphibian or reptilian lipoproteins is that in adult and tadpole bullfrogs (88, 93). Phosphatidylcholine was the major species in all lipoprotein classes, representing from 46.8% of the total in LDL from the adult to 75.9% in its HDL. Sphingomyelin was also present as a major component (17–41% in adult VLDL and LDL, respectively). Small amounts of phosphatidylethanolamine (trace—6%) and lysophosphatidylcholine (1.7–12.7%) were also detected. Bullfrog lipoproteins do not therefore appear to differ significantly in phospholipid distribution from those of man (Table 2 and ref. 44). The fatty acid compositions of the lipid esters of bullfrog and water snake lipoproteins are summarized in **Table 11;** the fatty acid pattern of a given lipid ester was essentially the same in all three bullfrog lipoproteins, with the exception of the sphingomyelin.

Although reptiles are also poikilothermic, the fatty acid patterns of their LDL lipids tended to be distinct. Thus, the bullfrog resembled man in that oleate and linoleate were amongst the major fatty acids in its cholesteryl esters, with small amounts of arachidonate also present; the only differences of note were that oleate predominated in the amphibian's esters while linoleate dominates in man (65), and that high levels of palmitoleate were present in bullfrog. By contrast, the cholesteryl ester fatty acid pattern of water snake LDL was highly unsaturated, apparently lacking any saturated acids and being rich in arachidonate (50%) and linoleate (27%). The water snake would therefore appear more closely related to the fishes in possessing such a long chain unsaturated acid content in its cholesteryl esters, although as previously noted, 20:4 acid was present to a variable degree in the lipoprotein lipids of the various groups of fish. The cholesteryl esters of water snake lipoproteins thus appear intermediate in composition between those of the fish and the higher vertebrates, i.e., the birds and mammals. Whether the cholesteryl ester fatty acid pattern in reptiles and amphibians results from the specificity of LCAT itself in these species, or is a direct function of the fatty composition of its substrate lecithin as suggested by Gillett (94) awaits assessment.

Some resemblance was seen in the triglyceride fatty acid patterns of bullfrog and water snake lipoproteins, in which case oleate was, as in man, the major component. The similarity between the human, amphibian,

TABLE 11. Fatty acid compositions of the lipid esters of amphibian and reptilian lipoproteins

Species	(Ref.)	Lipoprotein Fraction	Lipid	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:4	22:0	24:1
									%						
Bullfrog	(88)	VLDL	Cholesteryl ester	trace	7.7	20.7	1.4	57.2	8.8			4.1			
Bullfrog	(93)	LDL	Cholesteryl ester	trace	8.3	17.8	trace	45.2	16.1			8.1	8.0		
Water snake	(6)	LDL	Cholesteryl ester					19.7	27.1				50.4		
Bullfrog	(93)	HDL	Cholesteryl ester	trace	8.5	15.8	1.7	54.8	12.3			4.8	1.8		
Bullfrog	(93)	VLDL	Triglyceride	3.8	28.9	20.4	3.4	37.1	5.0			1.5			
Bullfrog	(88)	LDL	Triglyceride	3.5	26.1	18.5	3.2	32.3	11.8			4.7			
Water snake	(6)	LDL	Triglyceride		11.1	2.4	5.4	36.1	18.5				12.4		
Bullfrog	(93)	HDL	Triglyceride	3.6	30.3	21.2	2.5	33.2	7.7			1.2			
Water snake	(6)	LDL	Phospholipid		5.0		17.0	20.0	18.0				34.2		
Bullfrog	(88)	VLDL	Phosphatidylcholine	0.9	60.6	1.0	4.2	28.9	4.3						
	(93)	LDL	Phosphatidylcholine	0.9	44.1	7.8	4.9	19.1	9.4			2.6	1.4		
	(88)	HDL	Phosphatidylcholine	3.0	46.4	9.5	4.9	28.9	4.9			2.0			
	(88)	VLDL	Sphingomyelin	trace	61.2	trace	4.1	2.1			2.8			4.1	25.8
	(93)	LDL	Sphingomyelin	1.0	68.6		2.9	5.3	0.9		0.9			2.2	14.5
	(88)	HDL	Sphingomyelin	1.6	54.9	4.7	4.8	21.4	2.9		trace			1.0	8.2

and reptilian triglycerides also extended to the presence of moderate to high levels of palmitic and linoleic acids. The bullfrog could be distinguished by its elevated palmitoleate content (18.5–21.2%). A modest level of arachidonate was detected in the snake (LDL) triglycerides, a substantially greater proportion of this acid being present in its phospholipids; indeed, it was the major component (34%). Large amounts of the 18:0, 18:1, and 18:2 acids (17–20%) were also found in the snake phospholipids, a characteristic shared by its human counterparts (88). The two major phospholipid species of bullfrog lipoproteins, i.e., phosphatidylcholine and sphingomyelin, were highly saturated, palmitic acid representing some 45% or more (range 44–69%) of the total in each case. Oleic acid was also present in elevated amounts in the phosphatidylcholine of each bullfrog lipoprotein class, a property shared only by the sphingomyelin of its HDL. The fatty acid pattern of this latter phospholipid is particularly noteworthy since it was the only lipid ester of the amphibian's lipoproteins to display significant proportions (8–26%) of a long chain unsaturated fatty acid, notably of 24:1.

Thus, the bullfrog, despite its cold-blooded nature, does not exhibit the same characteristic as the fishes in requiring high proportions of polyunsaturated fatty acids in its lipid esters. Nevertheless, the proportion of saturated fatty acids in its cholesteryl esters (10%), triglycerides (30%), and phospholipids (50–70%) was rather similar to that seen in certain fish species, such as the shark, *Centrophorus squamosus* (57), in which the saturation of CE 1 was 15–20%, of triglycerides 20–25%, and phospholipids 40–45%. The water snake appears to have conserved more of the typical properties of fish lipoprotein lipids than the bullfrog, but whether this results from dietary fatty acid intake or is the reflection of a direct structural requirement remains to be assessed. Finally, it is noteworthy that the role of the cholesteryl esters of both amphibian and reptilian lipoproteins seems to differ from that of the fish, since the esters of the former do not appear to function in the transport of the essential fatty acids, eicosapentaenoate and docosahexanoate. The above data are however drawn from studies of only a single species of amphibian and reptile, and should be extended to other members of these vertebrate classes before more general conclusions may be drawn.

Data on the protein moieties of the serum lipoproteins in these oviparous vertebrates is at most fragmentary. However, biochemical and immunological evidence has been obtained for the presence of a counterpart to human apolipoprotein B in the LDL of a reptile, the rat snake (80). This protein of high molecular weight (>250,000), was separated by gel filtration chromatography in detergent solution and was found to exhibit an amino acid profile similar to that found in man, other mammals, birds, and fish. The major protein component of bullfrog LDL, delipidated in deoxycholate and separated from low molecular weight peptides and lipids by gel filtration chromatography (93), also exists as a high molecular weight protein, since it eluted in the void volume of a Sephadex G-200 column. The amino acid composition of this material corresponded well to that typical of apo-B in a wide range of vertebrates (80), being enriched in aspartic and glutamic acids and leucine, and poor in methionine, proline, and histidine. These observations of Suzuki et al. (93) raise the possibility that an apo-B-like component is present in amphibians as well as in reptiles (80). Electrophoretic examination of bullfrog apo-VLDL in SDS-polyacrylamide gels failed to demonstrate this component however, prob-

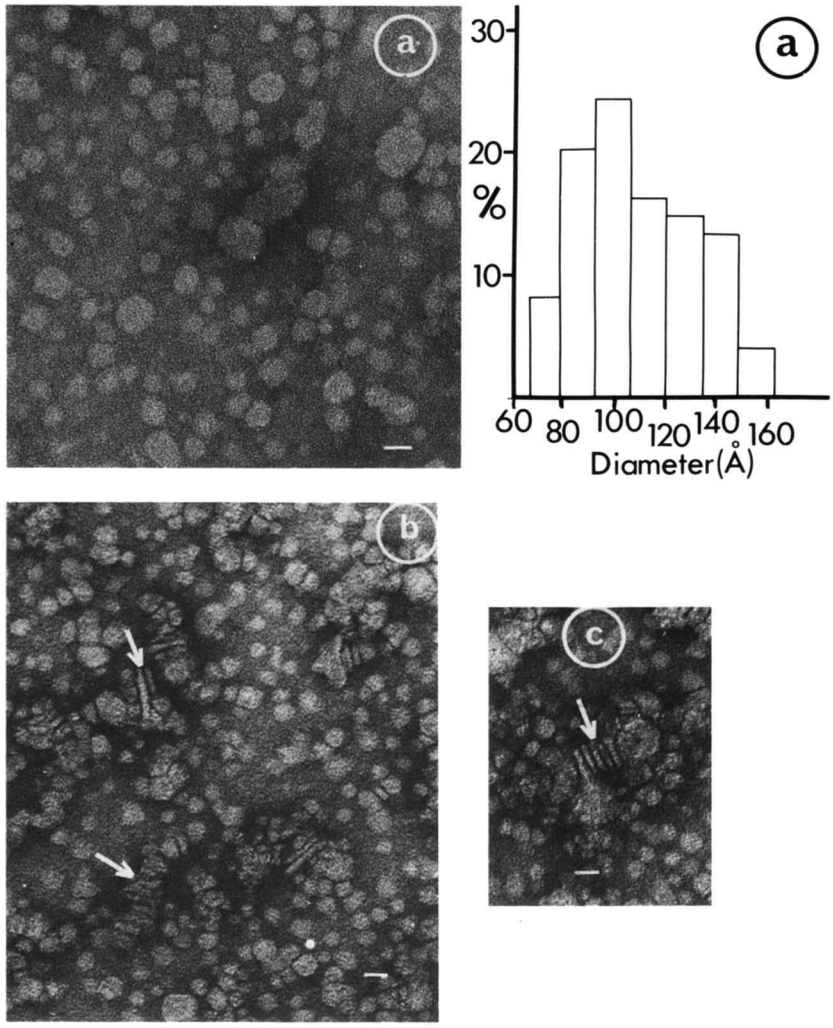

Fig. 4. Electron photomicrographs and particle size distributions of salamander (*Pleurolides Waltii*) HDL (d 1.063–1.21 g/ml) after negative staining with 2% phosphotungstate. Each bar represents 500 Å. a), when examined at low concentration (~0.15 mg protein/ml), the particles appear spherical, and are highly heterogeneous in diameter as illustrated by the frequency distribution of particle sizes shown at right. In this fraction, the modal and mean diameters were 106 and 111 Å respectively. This distribution excludes up to 5% of the occasional particles detected at either extreme of the range. b) and c), when examined at higher concentration, salamander HDL appears as both spherical and discoidal particles; arrows indicate disc-like particles in stack formation.

ably since gels of high monomer concentrations were used (10 and 20%). Proteins of molecular weight greater than about 100,000 penetrate these gels to a minimal extent. Thus, the major peptide components identified in apo-VLDL in 10% gels were of 7,000 molecular weight, corresponding probably to the human C proteins (2–4). Small amounts of these polypeptides were detected in bullfrog apo-HDL, but in this case the major protein was of 28,000 molecular weight, in all likelihood, a counterpart to human apo-AI.

The physical properties of reptilian and amphibian lipoproteins are as yet unexamined, although bullfrog and human LDL appear alike in molecular weight (2×10^6 (93) and $2.2–3.5 \times 10^6$ (4)). Preliminary examination of negatively-stained salamander (*Pleurolides waltii*) HDL by electron microscopy (**Fig. 4a,b** and **c**) suggests this fraction to be rather heterogeneous, containing particles with diameters larger (range 65–180 Å) than those typical of human HDL_2 and HDL_3 (mean diameters 95 and 75 Å, respectively) (4). Moreover salamander HDL tends to form stacks, or rouleaux, in crowded fields (Fig. 4b and c); these discs ranged from 150–400 Å in width and were about 50 Å in thickness. We have yet to determine the relative contributions of the normal, spherical type of HDL and of the discoid, vesicular-type particle to these fractions.

The above investigations of the serum lipoproteins

in amphibians and reptiles are especially subject to criticism on the grounds that details of the animals from which blood was obtained were meagre (5, 6, 88, 91); often, only one specimen of each species has been studied. The limitations of such an approach are aptly illustrated by the study of Gillett and Lima (95), who clearly established that a marked diminution in serum cholesterol level occurs with increasing age and change in diet in the male iguanid lizard, *Tropidurus torquatos*. Such findings suggest age and diet to be an important factor in regulating lipoprotein concentration in these reptiles. Marked differences were also evident between males and females. Further work will however be needed to determine whether such variations are related to vitellogenesis in the female.

The role of serum lipoproteins in oogenesis in amphibians and reptiles has been neglected, even though they are oviparous vertebrates and at least one of them, the toad *Xenopus laevis*, has served as a model for study of the relationship between plasma vitellogenin and the egg yolk proteins, lipovitellin and phosvitin (96). Present knowledge of amphibian plasma vitellogenin does however indicate it to be a lipophosphoprotein complex, with a lipid content (primarily phospholipid with small amounts of neutral lipid) of 12% and partial specific volume of 0.74 (97). The hydrated density of 1.35 g/ml of the complex would therefore correspond to a VHDL, although its function and protein components are quite distinct from those of this class of lipoprotein in man.

Birds

Of the oviparous vertebrates, the lipid transport systems of avian species have received by far the most attention. Such a degree of interest is accounted for by the use of several avian species (especially the chicken, *Gallus domesticus*) as animal models in which to study estrogen-induced hyperlipidemia (98–100), diet-induced hypercholesterolemia (99, 101, 102), and the molecular mechanisms involved in lipoprotein (VLDL) synthesis (103–105). In addition, the spontaneous susceptibility of certain breeds of pigeon (White Carneau, Columba sp.) to atherosclerosis and the relative resistance to this disease in others (Show Racer) has led to the utilization of this species by investigators of atherogenesis (106, 107).

The metabolism of lipids in birds differs from that in man in two important respects. First, avian species absorb exogenous fat via the portal system as large VLDL or "portomicrons", rather than as chylomicrons (and VLDL) via the lymphatic system as typically occurs in mammals (108). The second major difference between birds and many mammals is that the liver is the major site of lipogenesis in avians, whereas lipid biosynthesis occurs both in liver and adipose tissue in mammals (109). These dissimilarities are primarily quantitative in nature, since the pathways of intermediary metabolism in avians qualitatively resemble those of man (109); nevertheless, differences of this nature might be manifested in contrasting modes of lipid transport. In addition to these factors is the involvement of certain serum lipoproteins as precursors of egg yolk lipid in birds (110, 111), a role that may lead to important perturbations of serum lipoprotein profile during periods of active egg formation.

There have been numerous reports of the qualitative and quantitative aspects of the serum lipoproteins in avian species, primarily in the chicken (*Gallus domesticus*), but also in the turkey (*Meleagris galapavo galapavo*) the goose (*Anser anser*), the pigeon (Columba sp.) and the quail (*Coturnix coturnix japonica*). However, despite the recognized role of estrogens as activators of VLDL synthesis and secretion in birds (103–105), as well as in fish (76, 78), the relationship between serum lipoprotein profile and sex, age, and hormonal status has only been superficially evaluated, by Yu, Campbell, and Marquardt (112) in the chicken. These authors examined the distribution of plasma lipoproteins in mature, single comb White Leghorn hens and roosters. Levels of VLDL and HDL were rather alike in the rooster and non-laying hen (VLDL, 54 and 71 mg/100 ml plasma and HDL 370 and 361 mg/100 ml, respectively). In contrast, VLDL was elevated and HDL diminished in the laying hens (1401 and 151 mg/100 ml, respectively). LDL concentrations varied less, being similar in laying and non-laying birds (respectively, 152 and 183 mg/100 ml) and lower in the rooster (86 mg/100 ml). These results are generally consistent with others in the literature. Thus, the 4 week- and 8 week-old cockerels (New Hampshire Columbian cross) of Kruski and Narayan (113) displayed LDL and HDL levels (41–64 and 268–338 mg/100 ml serum, respectively) only slightly below those found by Yu et al. (112). In an earlier study in similar animals (101), the former authors observed similar HDL levels (354 mg/100 ml serum) and higher concentrations of LDL (d 1.019–1.063 g/ml:81 mg/100 ml serum). In both reports however (101, 113), Kruski and Narayan found VLDL levels markedly lower in young New Hampshire cockerels (7–17 mg/100 ml serum) than in the mature White Leghorn roosters (54 mg/100 ml plasma) examined by Yu et al. (112); such differences could be related to the transition in lipoprotein profile which is known to occur during development in other birds (107), mammals (114), and man (115). The lack of VLDL in the serum of adult roosters has also been documented by Fried et al. (116) and by Hillyard, White, and Pangburn (117),

the former authors noting the presence of LDL and HDL in amounts (104 and 385 mg/100 ml plasma, respectively) resembling those described above.

Some disparity thus exists in VLDL levels seen in roosters. In addition, reported concentrations of HDL in laying hens vary widely, 78–150 and 634 mg/100 ml serum reported by Chapman, Goldstein, and Laudat (118) and Hillyard et al. (117), respectively.

The initial finding of Schjeide (119) that the low VLDL levels of immature chickens could rise dramatically to reach concentrations of the order of 1000–2000 mg/100 ml serum with the onset of egg production has subsequently been confirmed by several investigators (112, 117, 120); similar or even higher levels may be attained in males upon estrogen (e.g., diethylstilbestrol) treatment (99, 104). The VLDL of laying hens primarily consists of small particles, range 270–540 Å, mean diameter 340 Å (118), of low flotation rate (i.e., S_f 20–100, (117, 118)), with only small amounts (approximately 25% of the total) of higher S_f, i.e., 100–400. Such a skewed distribution is not only characteristic of the VLDL in laying hens, but also of their LDL. Thus the low density substances in these birds are principally of high flotation rate (S_f 12–20; peak S_f 11–13; (117, 118)), thereby resembling intermediate density lipoproteins (IDL) rather than the major LDL species (S_f 0–12, peak S_f 4–8) typical of mammals and man (6).

As Mills and Taylaur (6) originally pointed out, the high flotation rate seen in the LDL of *G. domesticus* is also typical of other avian species, including goose, pigeon, turkey, and quail; values for the peak flotation rate in the S_f range 0–12 for these species ranged from 11.9 to 12.7 (6). However, in none of these birds was the sex, age, or nutritional state known at the time of blood withdrawal, consequently limiting interpretation of the data (6). Furthermore, some dissimilarities are evident upon comparison of the profiles found in both turkey (*Meleagris galapavo galapavo*) and pigeon (Columba sp.) by the latter workers (6) and by others (106, 120). Thus in fasted, male Broad Breasted White turkeys, Kelley and Alaupovic (115) found trace amounts of VLDL, while Mills and Taylaur (6) detected levels (2260 mg/100 ml serum) similar to those typical of laying chickens. Concentrations of LDL in the two studies were comparable (49 and 87 mg/100 ml serum in (6) and (120), respectively); HDL levels in male turkeys (some 320 mg/100 ml (120)) approached those of roosters and non-laying chickens (101, 112, 113, 116).

Similarly, moderate amounts of VLDL (146 mg/100 ml serum) and elevated quantities of both LDL and HDL (350 and 1352 mg/100 ml serum, respectively) were seen in the pigeons (*Columba palumbus* p.) examined by Mills and Taylaur (6), while Langelier, Connelly, and Subbiah (106) were unable to detect VLDL in fasting male pigeons (either White Carneau or Show Racer; *Columba livia*) and noted modest to elevated levels of LDL and HDL (115–191 and 500–557 mg/100 ml plasma, respectively) in both breeds. It is difficult to account for such differences, since details of Mills and Taylaur's birds (6) were not supplied.

In the only reported data on the serum lipoproteins of the goose and quail (6), the profiles were again typical of the birds since the distributions of both their VLDL and LDL classes were skewed in the direction of particles with high flotation rates. Lipoprotein concentrations themselves were low and in the range of about 50–100 mg/100 ml serum each for VLDL and LDL; HDL levels were elevated in the goose (761 mg/100 ml) but not as high as in the pigeon (1352 mg/100 ml).

While much of the above data in birds was obtained by analytical ultracentrifugation, other methodologies such as electrophoresis, have been used to analyze their lipoprotein profiles. As has been noted earlier in the trout (60), the electrophoretic mobilities of animal lipoproteins do not necessarily correspond to those characteristic of human lipoproteins (7). This is the case in the chicken for example, in which the anomalous mobility of isolated VLDL, LDL, and HDL has been documented (112, 113). Some caution should therefore be exercised in examining quantitative data on lipoprotein profiles obtained by such techniques unless otherwise validated. The semi-quantitative data obtained upon agarose gel electrophoresis of pheasant, duck, chicken, goose, and turkey sera (92) are generally consistent with the main features of the lipoprotein pattern in avian species determined by analytical ultracentrifugation, i.e., a predominance of HDL in immature and mature male birds and in non-laying females, with moderate levels of LDL and lesser (or even trace) amounts of VLDL. In laying birds, high concentrations of VLDL (>1000 mg/100 ml serum) typically occur in association with diminished amounts of HDL, whose levels may be similar or less than those of LDL.

In addition to the major lipoprotein classes in birds, a lipophosphoprotein occurs in the circulation of egg-laying females. This complex has been identified in chicken plasma (121), and is a vitellogenin, being dissociable into phosvitin and lipovitellins (122). It also contains variable proportions of β-lipoprotein (i.e., LDL), which may be present as a result of the precipitation procedure used in its isolation (122). As in amphibians, the vitellogenin complex apparently corresponds to a VHDL, since on the basis of a 15–20%

TABLE 12. Chemical compositions of VLDL and LDL from avian serum (% by weight)

Animal (Reference)	(6)	VLDL: Chicken: Immature Male (101)	Chicken: Rooster (112)	Chicken: Non-laying Hen (112)	Chicken: Laying Hen (112)	Chicken: Laying Hen (124)	Turkey: Adult Male (120)	Goose (6)	Pigeon (6)
Cholesteryl esters	17.5	41.3[ab]	9.3[a]	11.4[a]	5.1[a]	2.9[d]	26.6	24.2	27.2
Free cholesterol	5.3					5.4	3.7	4.8	8.1
Triglyceride	52.5	28.6	57.4	55.7	56.5	61.3	34.2	52.4	38.0
Phospholipid	17.9	19.1	18.5	18.6	25.8	30.7	21.7	9.2	18.8
Protein	6.8	10.9	14.8	14.3	12.7	14.0	13.8	9.2	6.8

[a] Cholesterol determined as total sterol.
[b] VLDL, d < 1.019 g/ml; LDL, 1.019–1.063 g/ml. Calculated from reference 101.
[c] Density 1.024–1.045 g/ml.
[d] VLDL, d < 1.010 g/ml; LDL, 1.01–1.06 g/ml. Triglyceride represents total of tri-, di-, and monoglycerides. Hydrocarbon content omitted.
[e] Turkey LDL fractions are 1) LDL, d 1.006–1.063 g/ml, and 2) LP-B and 3) LP-A, both isolated from an LDL fraction by affinity chromatography on concanavalin A-Sepharose 4B.
[f] W.C., White Carneau; S.R., Show Racer.
Unless otherwise stated, LDL fractions are of d 1.006–1.063 g/ml.

lipid content (118, 119), its hydrated density would be in excess of 1.21 g/ml.

As in man and other mammals, the chemistry, structure, and metabolism of the circulating low density (i.e., VLDL and LDL) lipoproteins of birds tend to be more closely related to each other than to those of the high density substances. The chemical and physical properties of avian VLDL and LDL will therefore be considered together. Reported chemical compositions for the VLDL and LDL of chicken, goose, pigeon, and turkey are summarized in **Table 12.** In the absence of data on particle distribution, which in the case of VLDL may be extremely heterogeneous, it is difficult to perform a valid comparison of VLDL composition in different species. It is however apparent that all the chicken fractions with density less than 1.006 g/ml are triglyceride-rich, irrespective of sex or laying status; the same may be said of goose and pigeon VLDL. The elevated triglyceride and phospholipid and diminished cholesterol contents of VLDL in laying hens appear particularly characteristic. The high cholesterol content of the fraction isolated from immature cockerels by Kruski and Narayan (101) may be related to its IDL content, although this fraction displayed a higher proportion of cholesterol (41.3%) than the corresponding LDL (24.3%); further studies of VLDL distribution and composition during the course of development in cockerels thus appear necessary.

The presence of large amounts of phospholipid and protein (ca. 35%) in turkey VLDL suggests that they are predominantly small in size and of low flotation rate, a supposition consistent with their low peak S_f (S_f 33) (120). As could be predicted from their higher density, the protein content of the avian LDL was greater in all cases than that of VLDL. Despite similar proportions of protein to human LDL, the LDL of the chicken and goose differed significantly ($P < 0.02$) from that of man in displaying 20% or more of its weight as triglyceride (Tables 5 and 12). This increase appears to be at the expense of cholesteryl ester, since the total neutral lipid content (triglyceride and cholesteryl ester) in these birds (range 44–50% in LDL of d 1.006–1.063 g/ml) resembled that in man (49.2%; ref. 6). Large variations can be seen in the individual proportions of the neutral lipids (including cholesterol as total or as free and esterified) in the fractions, possibly a result of several factors, such as diet and differences in particle distributions. The importance of this latter element is well illustrated by examination of the chicken fraction isolated in a relatively narrow density interval (d 1.024–1.045 g/ml) (118); it is enriched in cholesteryl ester (34.9%) and resembles the corresponding human fraction (ester content 38.9%; ref. 86) more closely than the preparation of d 1.006–1.063 g/ml.

Turkey LDL (d 1.006–1.063 g/ml) was distinguished from the other avian LDL's by high cholesteryl ester and low glyceride contents, resembling the human fraction of d 1.024–1.045 g/ml in composition (86). Chromatographic separation and analysis of the major lipoprotein species in turkey LDL, i.e., lipoprotein B (LP-B), having (turkey) apolipoprotein B as sole protein component, revealed low proportions of both triglyceride and cholesteryl esters, and a protein content more characteristic of HDL than LDL (6, 120, 125). A second and minor lipoprotein species

LDL														
Chicken								Turkey[e]				Pigeon[f]		
								Adult Male					Adult Male	
(6)	Immature Male (101)	Rooster (112)	Non-laying Hen (112)	Laying Hen (112)	Laying Hen (118)	Laying Hen (118)	Laying Hen (124)	[1](120)	[2](125)	[3](125)	Goose (6)	(6)	W.C. (106)	S.R. (106)
27.8	[24.3[b]	[20.5[a]	[21.0[a]	[9.3[a]	34.9[c]	28.6	7.5[d]	38.2	14.6	28.4	34.9	29.8	[34.1	[37.6
6.4					13.4	8.3	10.9	5.1	10.6	8.7	8.1	10.0		
22.1	23.3	30.1	19.9	41.3	11.3	19.4	36.9	5.1	2.8	1.0	22.3	17.1	7.5	15.2
20.2	23.0	31.3	40.3	31.3	18.8	21.2	23.7	26.2	34.1	28.9	12.2	24.1	15.3	14.5
23.5	29.3	18.1	18.8	18.0	21.6	22.5	21.0	26.0	37.8	32.9	22.3	19.0	43.2	32.8

isolated from turkey LDL was lipoprotein A (LP-A), possessing (turkey) apolipoprotein A (7) as its sole protein component; this lipoprotein was richer in cholesteryl ester than LP-B but was similarly deficient in triglyceride. These data clearly illustrate the heterogeneity possible in LDL of density 1.006–1.063 g/ml. It is noteworthy that LP-B was the major lipoprotein species of the LDL class, and that its cholesteryl ester content (14.6% (125)) was less than half that of the total LDL density class (38.2% (120)); an explanation for this disparity is not readily apparent, although it might suggest wide variation in LDL particle composition between different donor turkeys.

The composition of LDL in pigeons varied the most widely of all the birds; that of the White Carneau breed was poor in triglyceride but exhibited the highest protein content (43.2%) of all the LDL's examined (106). Such an elevated proportion of protein is more reminiscent of a high ($d > 1.063$ g/ml) rather than of a low ($d < 1.063$ g/ml) density fraction (6). The LDL of White Carneau and Show Racer pigeons differed markedly in composition, warranting qualitative and quantitative studies of their constitutive particles.

Few data are available on the individual phospholipid species present in avian low density lipoproteins, the only analyses having been made in chickens (110, 124) and turkey (120). Phosphatidylcholine was the major component in both turkey VLDL and LDL, representing some 60% of the total; lesser amounts of sphingomyelin (22–28%), lysophosphatidylcholine (13%) and phosphatidylethanolamine (7–9%) were also detected. Similarly, lecithins were the major phospholipids (83–86% of the total) in the VLDL (110, 124) and LDL (124) of laying hen plasma, with phosphatidylethanolamine primarily accounting for the remainder. These phospholipid distributions closely resemble those of the human lipoproteins (Table 2 and refs. 44 and 65).

Gornall and Kuksis (110) have provided extensive information on the distribution of molecular species of both phospholipids (phosphatidylcholine and phosphatidylethanolamine) and triglycerides in VLDL from laying hen plasma: unfortunately, LDL and HDL lipids were analyzed together, rendering these results of limited value. The major species of VLDL triglyceride were dienoic and trienoic in nature with a total of 52 carbon atoms; their principal fatty acids were 16:0 and 18:1, and 16:0, 18:1, and 18:2, respectively. In contrast, monoenoic and dienoic species of 36 carbon atoms were the predominant types of phosphatidylcholine, while monoenes, dienes, and polyenes (primarily of 36–40 carbon atoms) represented the major species of phosphatidylethanolamine. Analyses of the total fatty acid composition in each phospholipid class showed slight differences in pattern, since an elevated proportion of polyenoic acids (20%, consisting of 20:4, 22:4, and 22:6) was present in VLDL phosphatidylethanolamine; palmitate, stearate, oleate, and linoleate were the dominant fatty acids of both phospholipid classes however. The major fatty acids of the VLDL triglycerides were the same as those of the phospholipids, i.e., 16:0, 18:0, 18:1, and 18:2. Comparison of the patterns of triglyceride fatty acids in chicken and human VLDL (44) revealed an overall likeness, that extended to their phospholipids; the only type of polyunsaturated acid

in human VLDL phospholipid, however, was arachidonate.

The distributions of fatty acids in the lipid esters of the LDL of the goose (5) and of Show Racer and White Carneau pigeons (106) have also been described. Overall resemblances were seen in the triglyceride fatty acid patterns of goose and human LDL, oleate predominating. Some similarity was also apparent in their phospholipid patterns, each containing elevated amounts of arachidonic acid; but where palmitate was the major fatty acid of human LDL phospholipid, it was only a minor component of the avian substances. Although oleate accounted for more than half of the cholesteryl ester fatty acids of goose LDL, it represented only 22% of the human compounds, in which linoleate usually represents 50% or more; the cholesteryl esters of the two species were alike however in transporting significant amounts (of the order of 1% of total fatty acids) of arachidonate.

The fatty acid patterns for all three lipid esters of pigeon LDL (106) varied little, the 16:0, 18:1, and 18:2 acids being generally present in elevated quantities (15% or more). Only minor differences could be detected between Show Racer and White Carneau breeds. As distinct from goose and human LDL, trace amounts of arachidonic acid were detectable in the lipid esters of pigeon LDL. These observations indicate marked differences in the mode of transport of essential fatty acids in avian species, though additional data will be required before any general conclusions can be drawn. The fatty acids of avian lipoproteins do however resemble those of the mammalian substances more than those of the fish, reptiles, and amphibians, since they contain only minor amounts of fatty acids greater than 20 carbon atoms in chain length.

Some basis exists for suggesting a common three-dimensional organization in avian (chicken) and human LDL, particularly upon consideration of the relatively homogeneous fraction of d 1.024–1.045 g/ml (118). The avian and human particles exhibited a similar morphology, although the diameter of chicken LDL was slightly greater (mean 234 Å; man, mean 217 Å) (118); this was probably due to the higher triacylglycerol content of the chicken particles. The total neutral lipid content of LDL in the two species was nevertheless almost identical (46.2% in chicken and 45.2% in man). This resemblance extends to the major protein component of LDL (ca. 80% of chicken apo-LDL) from the two species, since chemical, physical, and immunological evidence has been provided to indicate that the chicken protein is a counterpart to human apolipoprotein B (118).

Originally, Hillyard and colleagues suggested in 1972 (117) that the apolipoproteins of avian and mammalian lipoproteins might be not only immunologically but also chemically nonidentical. These authors found that about 90% of the VLDL and LDL apolipoproteins was a polypeptide of 21,500 molecular weight. Their finding could not be confirmed (103, 104, 118, 126), and indeed several investigators subsequently detected the presence of a major apolipoprotein of high molecular weight (and low solubility) in chicken VLDL (103, 104, 118, 126) and LDL (118) by various procedures, including gel filtration chromatography in detergent-containing buffers and SDS-polyacrylamide gel electrophoresis.

The apo-B-like nature of a protein component in chicken LDL was initially suggested to Goldstein and Chapman (127) by the immunological cross-reactivity of human and avian lipoproteins upon examination with heterologous and homologous antisera. Similarly, a reaction of partial identity was found between chicken and human VLDL (118). In addition, a common antigen was detected in chicken VLDL and LDL (118), a finding in accordance with the data of others (103, 104, 117, 126). Use of an antiserum to human apolipoprotein B then served to reveal a partial identity between the VLDL and between the LDL of the two species (118). Isolation from apo-VLDL and from apo-LDL of the high molecular weight protein (>250,000) responsible for such immunological activity was accomplished by gel filtration chromatography; comparison of their chemical and physical properties showed them to be indistinguishable in amino acid composition and behavior on SDS-polyacrylamide gels (118). Furthermore, the amino acid composition of these fractions was essentially identical to that reported by Chan et al. (104) for the high molecular weight fraction of chicken apo-VLDL, and in turn to that of human apolipoprotein B (86). Considered together, these data serve to establish the presence of a protein, analogous to human apo-B, as the major apolipoprotein of chicken VLDL and LDL. We have referred to this protein as chicken apo-B (118).

A counterpart to human apo-B has also been identified in turkey serum lipoproteins by Kelley and Alaupovic (120); this apolipoprotein occurred principally in the VLDL and LDL density ranges, but was also detectable in small amounts in HDL (120, 125). As in chicken and man, turkey apo-B was insoluble in aqueous buffers, but could be solubilized in guanidine-HCl after reduction and carboxymethylation; such treatment facilitated its isolation by column chromatography on Bio-Gel A-5m with 6M guanidine-HCl as the eluant (120). Again, this protein

eluted in the void volume with high molecular weight. Comparison of its amino acid profile with that of chicken and human apo-B (86, 118) revealed a marked degree of resemblance, with aspartic and glutamic acids and leucine as the major amino acids (>10%). Applying monospecific antisera to turkey lipoprotein B, Schjeide et al. (128) have demonstrated in immunological studies the common possession of an analogue to human apo-B in turkey and chicken lipoproteins; this apolipoprotein was also identifiable in serum from chicken embryos (128). Apo-B of turkey and man were also alike in possessing significant quantities of carbohydrate and, in both species, mannose, galactose, and galactosamine are the major components (2, 4, 120). Unlike the human protein however, turkey apo-B was deficient in sialic acid, although this sugar was detected among the carbohydrate residues of chicken serum apo-VLDL by Abraham, Hillyard, and Chaikoff (129). The exact nature of the high molecular weight apolipoprotein of avian lipoproteins, i.e., that termed apo-B, has recently become the subject of discussion following the observations of Williams (130). Such a situation is not confined to the avian protein however, a similar debate having surrounded the polypeptide subunit composition of human apo-B for many years (2, 4).

In parallel studies of VLDL isolated from diethylstilbestrol-treated roosters (130), the major apoprotein of plasma VLDL displayed a single band in SDS-polyacrylamide gel of 350,000 molecular weight, whereas the serum apoprotein showed a smaller amount of this component that was accompanied by about six bands ranging from 290,000 to 100,000; the 290,000 band predominated. Gel patterns of proteins smaller than 100,000 were essentially the same in the two preparations. Since an inhibitor of serine proteases was present during isolation of plasma VLDL, Williams (130) invoked proteolysis of apo-VLDL-B during its separation from serum to explain the presence of the additional bands in its SDS-gel pattern. Evidence from immunological analyses of serum apo-VLDL showed these bands (290,000 to 100,000) to be indistinguishable from the single high molecular weight component (i.e., of 350,000) of the plasma fraction, suggesting their derivation from it. Some question remains as to whether proteolysis is entirely responsible for Williams' findings (130). Thus direct evidence for proteolysis is needed, and it should be shown that the presence of protease inhibitors during isolation of serum VLDL (and LDL) preserves the 350,000 band as the sole high molecular weight component of its apoprotein. Such an approach was adopted by Chapman and Kane (83) when demonstrating the absence of endogenous proteolytic activity in the apoprotein of LDL isolated from human serum or plasma.

The low molecular weight components, tetramethylurea-soluble apolipoproteins, of avian VLDL and LDL are distinct from those of the human substances. Thus there appears to be essentially one major low molecular weight polypeptide in the VLDL of laying hens and estrogenized roosters (104) together with up to six minor components (118). The sum of these polypeptides represented 50–55% of the total protein moiety of VLDL (104, 118, 130). The major apolipoprotein, termed fraction B or apo-VLDL-II by Chan and colleagues (104, 105, 131), constituted approximately 85% of these peptides (104). The amino acid sequence of apo-VLDL-II has recently been reported by Jackson et al. (131); it has a molecular weight of 9,400 and is normally present as a dimer of two identical polypeptide chains, each of 82 amino acid residues. These chains are joined by a single disulfide bond at residue 76. Comparison of the amino acid sequence of chicken apo-VLDL-II with that of apovitellenin I, purified from the yolk low density lipoproteins of chicken (132), emu (133), and duck (134) egg yolks has revealed considerable homology between them. It is especially interesting that the highly polar (residues 5–10) and highly hydrophobic (residues 11–23 and 33–48) regions of these four proteins have been highly conserved (131–134). Such findings clearly implicate chicken apo-VLDL-II in egg formation, a conclusion consistent with the direct uptake of serum VLDL by the developing ovary (11). Certain of the protein components of avian lipoproteins therefore seem to function specifically in egg yolk formation, and indeed may carry "recognition sites" which trigger uptake by oocyte receptors. Nevertheless, avian low density lipoproteins still appear to need structural proteins, such as apo-B, for their stabilization; the role, if any, of this protein in egg formation remains conjectural (135).

Several investigators have documented the distinct properties of the high-density class of lipoproteins in avian serum and their analogy to the HDL of mammals and man (120, 136). A summary of the chemical compositions of HDL from birds is presented in **Table 13;** analyses lacking determination of cholesteryl ester, a fundamental serum lipoprotein constituent, have been omitted. The HDL of all the avian species is characterized by higher protein content (range 43.9–64.0%) and consequently lower proportions of lipid than LDL (Table 12), thus conforming to the general concept that as hydrated density increases, the ratio of lipid:protein in these macromolecules diminishes. The proportion of triglyceride is uniformly less

TABLE 13. Mean weight percent chemical composition of HDL from avian serum

	Chicken[a]				Turkey[b]					Human[c]	
		Laying Hen	Immature Cockerel	Rooster	Adult Male			Goose	Pigeon		
(Reference)	(6)	(124)	(101)	(136)	(120)	(125)	(125)	(6)	(6)	(136)	(136)
					1)	2)	3)			1)	2)
Cholesteryl ester	20.4	7.9	15.0	16.0	8.7	14.5	13.2	19.2	18.5	16.2	11.7
Free cholesterol	2.2	1.4	2.9	5.0	2.9	1.1	9.5	2.0	2.5	5.4	2.9
Triglyceride	7.4	8.2	2.0	6.4	3.5	0.5	2.9	8.8	6.5	4.5	4.1
Phospholipid	22.3	17.6	24.0	28.7	26.4	29.5	32.1	17.5	25.6	29.5	22.5
Protein	47.8	64.0	56.1	43.9	58.5	54.5	42.2	52.6	46.8	41	55

[a] Density 1.06–1.20 g/ml; hydrocarbon content < 1%.
[b] Turkey HDL fractions are 1) HDL, d 1.063–1.21 g/ml, and 2) LP-A and 3) LP-B, both isolated from total HDL by affinity chromatography (125).
[c] Human HDL fractions are 1) HDL_2, d 1.063–1.125 g/ml and 2) HDL_3, d 1.125–1.21 g/ml.

in avian HDL than in the corresponding LDL fractions; the same is true for free cholesterol.

Comparison of avian HDL with human HDL_2 and HDL_3 subfractions suggests that changes in overall particle distribution may occur during maturation. The composition of HDL in immature cockerels (101) resembled that of human HDL_3, while that of the rooster (136) was more closely related to the HDL_2 subfraction, especially in protein content. No clear relationship was apparent between the chicken, goose, and pigeon HDL analyzed by Mills and Taylaur (6), since the neutral lipid contents of these fractions (27–28%) were largely in excess of those of either HDL_2 (21%) or HDL_3 (16%) of man. Finally the ratio of esterified to free cholesterol in avian HDL (EC/UC; 4–6:1) was greater than that seen in the HDL of reptiles and fish, and thus more closely resembled the human compounds (EC/UC ca. 3.0).

Turkey HDL (fraction (1), Table 13; ref. 120, 125) and human HDL_3 were alike in composition, an observation inconsistent with the analytical ultracentrifugal profile of the turkey fraction which displayed a single, symmetrical peak at d 1.21 g/ml with a flotation coefficient of 4.2 (120), thereby resembling HDL_2 more than HDL_3 (65). The major lipoprotein family of turkey HDL was lipoprotein A (fraction (2), Table 13); this particle contained rather more cholesteryl ester than the whole fraction of 1.063–1.21 g/ml, but was otherwise similar in composition. The minor component, lipoprotein B, resembled human HDL_2 more than HDL_3, and like LP-A, was richer in cholesteryl ester than the 'parent' fraction. The observation that the cholesteryl ester content of the 'parent' HDL (fraction (1), Table 13) is lower than that of both its constitutive particle fractions, LP-A and LP-B, is difficult to explain, but since the latter fractions were isolated from a different batch of serum (125) to whole turkey HDL (120), then it could be accounted for by wide variation in the composition of HDL particles between different turkeys. A similar argument was proposed to explain discrepancies between the cholesteryl ester contents of turkey LDL (120) and of its constitutive particles, LP-B and LP-A (125) (see Table 12).

The distribution of phospholipids in avian lipoproteins, though only poorly studied, appears to bear an overall resemblance to that of their human counterparts (65, 44, 120, 136). Thus phosphatidylcholine was the major species of rooster VLDL and LDL (136), and of rooster and turkey HDL (120, 136). As in human lipoproteins, sphingomyelin constituted some 20–27% of avian (turkey) VLDL and LDL, but less of HDL (turkey, 12.8%; rooster 8.0%). Phosphatidylethanolamine and lysophosphatidylcholine were the minor components in all cases.

Data on the fatty acid composition of avian lipoprotein lipids is limited to the LDL and HDL of two breeds of pigeon (106) and to the HDL of the rooster (136). The fatty acid patterns in the lipid esters of the LDL of each breed of pigeon did not differ with the exception of a lower stearate content in LDL triglyceride of the White Carneau. The composition was similar to that of human LDL (44, 65). Similar comments are applicable to the fatty acid patterns of pigeon HDL lipids and to those of rooster HDL triacylglycerols, cholesteryl esters, phosphatidylcholines, phosphatidylethanolamines, sphingomyelins, and lysolecithins. Only the greater proportion of 18:0 in the avian phospholipids (except sphingomyelin) and triglycerides is worthy of comment. The role of both avian and human HDL phosphatidylethanolamine in the transport of long chain polyunsaturated fatty acids (20:4 and 22:6) is notable (136). Of the nonmammalian vertebrates, the apolipoproteins of avian HDL are the most extensively investigated to date. It may therefore be said with some degree of certainty that the major apolipoprotein of both chicken and turkey HDL is homologous with human

apo A-I. Data on the purified chicken protein have been reported by Jackson et al. (138) and by Kruski and Scanu (136), and on the turkey protein by Kelley and Alaupovic (120).

Chicken and turkey apolipoprotein A-I have each been isolated and purified from either delipidated HDL (rooster (136) and chicken (138)) or HDL_3 (turkey (120)) by gel filtration chromatographic techniques, involving use of strong dissociating agents such as concentrated urea (136, 138) or guanidine-HCl solutions. In some cases, rechromatography was necessary in order to obtain the pure apolipoprotein (120). Estimates of the molecular weight of this protein vary according to the method employed; by SDS-gel electrophoresis, hen A-I had a molecular weight of ca. 28,000, whereas its size, calculated from amino acid analyses (234 assumed amino acids), was 26,674 (138). The rooster protein was judged slightly smaller, ca. 26,000, by electrophoresis in SDS-polyacrylamide gel (136); the molecular weight of turkey A-I was estimated to be 27,000 (120). These values closely approximate that of human apo A-I (28,331 on the basis of amino acid composition (4)). Similarities with the human protein also include migration in basic urea-polyacrylamide gel (turkey (120), rooster (136)), and the absence of a carbohydrate moiety (turkey (120), man (3)).

Comparison of the N- and C-terminal sequences of turkey (120), chicken (138) and human apo A-I (3) reveals only a minor degree of sequence homology. Indeed even the carboxyl-terminal residue of turkey A-I (alanine) differs from that of man (glutamine), but is the same as that of rat A-I (139). Furthermore both chicken and turkey A-I differ from the human protein in containing isoleucine. Consistent with such dissimilarities, immunological analyses of hen and human A-I, using antisera to the chicken protein, revealed a partial identity (138). Further consideration will be given to the N- and C-terminal sequences of animal apo A-I's later in this article (see Fig. 5). A protein migrating in urea-polyacrylamide gel to a similar position as human A-II has also been detected in turkey apo HDL_3 and in rooster apo HDL (120, 136). Thus, in both rooster apo HDL and turkey apo HDL_3, a band of greater mobility than the A-I component was noted in basic urea-polyacrylamide gel (120, 136); this protein comprised about 10% of total rooster apo HDL, was unaffected in its migration by reducing agents, and had a molecular weight of about 15,000 as judged by SDS-polyacrylamide gel electrophoresis.

Kelley and Alaupovic (120), having identified two antigenic components in turkey apo HDL_3 by immunochemical analyses, were successful in purifying the minor apolipoprotein from apo HDL_3 by gel filtration chromatography in guanidine-HCl. The molecular weight of this protein was 10,000 and it was designated turkey A-II. Monospecific antisera to turkey A-II showed its non-identity with the A-I component. Turkey A-II resembled the human protein in having a blocked N-terminus, in lacking a carbohydrate moiety, and in possessing lysine, glutamic acid, and leucine as its major amino acids. The avian apolipoprotein was however distinct from its human counterpart in molecular weight, in lacking cystine, in containing histidine and arginine residues that are absent from the human protein (4), and in existing in monomeric form. Some question remains as to whether apolipoproteins equivalent to the C-peptides of man exist in avian VLDL (118) or HDL (117, 120, 136); if present, they occur in trace amounts.

The chemical properties of the lipid and protein moieties of avian HDL may therefore be said to resemble those of the human compounds to a marked degree. Do the avian substances then display physical properties which would suggest a similar structural organization to their human counterparts? The answer to this question is paradoxical and based only on the observations of Kruski and Scanu (136). Thus the chemical composition of rooster HDL resembles that of human HDL_2 while its molecular weight, partial specific volume, and frictional ratios are essentially identical to those of human HDL_3; its sedimentation and diffusion coefficients are distinct. These findings can only be interpreted as being indicative of some essential difference in structure between human and avian HDL.

In summary, the preceding survey indicates avian species form a rather specialized group within the oviparous vertebrates. Thus, not only do they utilize VLDL to assimilate exogenous lipids, but they have the ability to increase the synthesis and circulating concentrations of this lipoprotein class dramatically in response to demands for egg formation. It is thus apparent that the lipid requirements of the developing ovary can no longer be satisfied by the vitellogenins, (taking the form of high or very high density lipoproteins of relatively low lipid content), which fulfil this role in the invertebrates. Further investigations are clearly needed to delineate the roles of the very low density lipoproteins in the other three groups of oviparous vertebrates, fishes, amphibians, and reptiles, in which high levels of VLDL (with the exception of Cyclostomes, Elasmobranchs, Crossopterygians and Actinopterygians of the order Chondrostei) are rarely attained. Moreover, specific attention should be given to the function of the serum apolipoproteins, since in some species (e.g., birds,

the chicken), they serve equally as structural components of serum VLDL and of egg yolk lipoproteins, as in the case of apo-VLDL-II, which appears identical to vitelennin I; in other species, such as the Teleostean fish for example, specific proteins distinct from the serum apolipoproteins are involved in vitellogenesis.

Mammals

To date, the serum lipoprotein systems of mammalian species have been the focus of attention of a large body of scientific investigators, the majority of whom have been concerned with the use of these animals in some aspect of atherosclerosis-oriented research. Indeed, activity in this field has given rise to a voluminous literature during the past 8 years or so. The literature up to the period 1974–1975 has been reviewed by Mills (5) and by Calvert (140) and some information is also available in Kane's more recent article (4). For present purposes, I propose to review data on mammalian lipoproteins which have appeared since 1975, linking it to previous observations when appropriate. Further, even though the "pathological" lipoproteins of mammals whose lipoprotein profiles have been perturbed by dietary or other means have attracted considerable interest, such investigations will be considered only in cases when they afford added interpretation of information applicable to lipoprotein structure in the "basal" state. For summaries of such work, the reader should refer to the reviews of Calvert (140) and of Mahley (141).

Criticisms similar to those leveled earlier at studies of other animal lipoprotein systems are also applicable to investigations in mammals, and many authors have failed to properly delineate the density characteristics of the major lipoprotein classes. This would appear to be of particular importance in mammalian species, since certain among them display rather aberrant lipoprotein and apolipoprotein distributions. For instance, large amounts of lipoproteins with structural and metabolic characteristics typical of HDL may be present at densities substantially lower than 1.063 g/ml; examples are the dog (142) and rat (140). Conversely, particles typical of the low-density class may be distributed up to densities within the interval occupied by HDL, as occurs in the guinea pig (89, 90).

Such anomalies have necessitated the supplementation or replacement of the classical sequential ultracentrifugal procedure for isolation of lipoproteins with techniques of higher resolutory power, as exemplified by electrophoresis on Pevikon (142, 143), precipitation with polyanions (144), or density gradient centrifugation (145, 146). Indeed, their application may on occasion facilitate isolation of a single, homogeneous (animal) lipoprotein species (144).

As may be evident from the above comments, one of the most complex areas of the mammalian lipoprotein density distribution concerns the region around the 1.063 g/ml "boundary." A number of discrete, distinct lipoprotein species may coexist in this portion of the spectrum and these include: *1*) LDL, having apo B as its major apolipoprotein; *2*) Lp (a), having apo B and the Lp(a) polypeptide (and possibly additional components) in the protein moiety; *3*) HDL_1, originally denoted as lipoproteins of S_f 0–3 (65) but highly variable in apoprotein content and density distribution according to species; and *4*) a species denoted as LDL-II by Mahley and colleagues, corresponding to Lp (a) in man immunochemically, containing apo B, apo E, and apo AI and detected to date only in the normal *Erythrocebus patas* monkey (147). Such points as these will be specifically addressed in relationship to individual species in the ensuing treatise.

At the outset, some diversity in the modes of lipid transport in mammals must be anticipated on the basis of their widely varying diets and digestive physiology. The mammals may thus be classified into herbivores (e.g., rabbit and cow), carnivores (e.g., dog and seal), and omnivores (e.g., man and baboon). The herbivores may themselves be divided into those with simple stomachs (e.g., rabbit), in which case digestion of lipids occurs in the small intestine as in other simple-stomached mammals, and those with complex stomachs, such as the cow, which hydrolyzes and assimilates its dietary lipids in the rumen. In the majority of herbivores, HDL is the major lipoprotein class (148), although there are certain classical exceptions to this, such as the guinea pig (89, 90) and camel (6). The herbivores do not therefore appear to have taken recourse to specialized lipoproteins; neither do they exhibit a marked relationship between their digestive physiology and serum lipoprotein profile. The carnivores also tend to exhibit elevated levels of HDL, which usually dominate their lipoprotein patterns; LDL concentrations are typically low and often less than 100 mg/dl of serum. Considerable variation is seen in the lipoprotein profile of omnivorous mammals, and particularly in certain species of non-human primates, although their LDL levels are often higher than those of most carnivores and herbivores.

Consideration of mammalian lipoproteins on a taxonomic basis is more informative, however, than that discussed above, and this approach has been adopted. The class Mammalia comprises three major subclasses, the Prototheria, or Monotremata, the Metatheria, or marsupials, and the Eutheria, or placental mammals.

Monotremes

Relatively few members of this primitive mammalian group are in existence, and of these, the duck-billed platypus and spiny anteater are the most notable. None have been the subject of lipoprotein investigation.

Marsupials

Data on the circulating lipoproteins of marsupials are limited, pertaining essentially to the opossum (*Didelphis virginiana*). The major lipoprotein class in the serum of this South American marsupial is HDL (660 mg/dl) as noted in the early study of Lewis, Green, and Page (149), and only small quantities of LDL (56–75 mg/dl) were detected by analytical ultracentrifugation. These observations are consistent with the lipid analyses of Dangerfield et al. (91) in six marsupial species, in which HDL tended to predominate, although relatively high lipoprotein (lipid) levels were detected. By contrast, a high LDL level was seen in the single opossum examined by Alexander and Day (92), and the ratio of LDL/HDL concentration was among the highest of a wide range of animals and markedly resembled that typical of man. Such inconsistencies clearly indicate the need for further investigation of the lipoproteins of marsupial species, and particularly of those from Australasia.

Placental Mammals

These are the higher mammals, and are typically divided into nine major orders, i.e., Insectivores (such as the mole, hedgehog and shrews), Rodents, Lagomorphs (rabbits and hares), Carnivores (including cats, dogs, bears, and seals) Cetaceans (whales and dolphins), Proboscideans (elephants), Artiodactyls or even-toed ungulates (cattle, sheep, pigs, deer, and camels), Perrisodactyls or odd-toed ungulates (horses, tapirs, and rhinoceroses), and Primates (including monkeys and apes).

Insectivores

Although Insectivores occupy a key position in the evolution of mammalian species, only the serum lipoproteins of the hedgehog (*Erinaceus europaeus*) have been examined to date (6). Levels of HDL (430 mg/dl serum) were some fourfold higher than of LDL (98 mg/dl); VLDL was present in trace amounts. As in man, the principal form of LDL was present as material of S_f 0–12. In contrast, the chemical composition of hedgehog LDL (d 1.006–1.063 g/ml) was distinct from human LDL, and from the other mammalian LDL analyzed by Mills and Taylaur (6), in its low protein (14.6%) and elevated phospholipid (30.4%) contents. Apart from this, the proportions of neutral lipids and the ester:free cholesterol ratio were not dissimilar from those of human LDL. The question as to whether some substitution of phospholipid for protein has occurred in hedgehog LDL merits additional evaluation. The high density (d 1.063–1.21 g/ml) lipoprotein in the hedgehog were similar to those of man in weight % composition, with the exception of elevated proportions of both esterified (as cholesteryl ester) and free cholesterol in the former. Nonetheless, the ratio of esterified:free cholesterol in the two fractions was alike (2.5 and 3.1 in hedgehog and human LDL, respectively).

Rodents

Members of the order Rodentia are among the most widely employed in studies of lipoprotein structure and metabolism. Thus, observations in the rat (*Rattus norvegicus*) have often preceded comparable findings in man. The circulating lipoproteins and apolipoproteins of the normal rat, together with those of the rabbit (Oryctolagus sp., order Lagomorpha), the dog (Canis sp., order Carnivora), the pig (*Sus domesticus,* order Artiodactyla) and rhesus monkey (*Rhesus macacus,* a nonhuman primate of the family Cercopithecoidea, Genus *Macaca*), could be said to be the most thoroughly characterized at the present time.

The serum lipoprotein profiles in a number of laboratory-bred, adult rodents, including the rat, the white mouse (*Mus musculus,* C3H strain) and Mongolian gerbil (*Meriones unguiculatus*), maintained in a nonfasting state (150), and in ten species of wild rodent (the vesper mouse, (*Calomys lepidus ducillus*), the Egyptian spiny mouse, (*Acomys cahirinis*), the prairie deer mouse (*Peromyscus maniculatus bairdii*), the collared lemming (*Dicrostonyx stevensoni*), the Norwegian lemming (*Lemmus lemmus*), the brown lemming (*Lemmus sibiriens trimucronatus*), the laucha (*Calomys callosus*), the northern red-black vole (*Clethrionomys rutilus dawsoni*), the tundra vole (*Microtus oeconomus macfarlani*) and the meadow vole (*Microtus pennsylvanicus tananaensis*) (151)) have recently been compared to that of man. Employing electrophoretic and immunological techniques, Johansson and Karlsson (150) and Galster and Morrison (151) noted marked qualitative and quantitative differences in electrophoretic pattern which showed no correlation with the taxonomic classification of the respective species. Moreover, little correspondence was seen between the mobility of individual rodent lipoprotein fractions, isolated by ultracentrifugal and precipitation procedures, with the corresponding human lipoproteins (150). In addition, large variations were seen in triglyceride and free fatty acid levels in the ten

wild species maintained under laboratory conditions (151), although cholesterol concentrations in eight of these were rather invariant and in the range 115–180 mg/dl serum.

Although not without interest, the above studies clearly illustrate the inadequacy of electrophoretic criteria in comparative studies of lipoprotein profile. As an example, the most aberrant of the species examined was the mouse (*M. musculus*), in which isolated HDL migrated to the albumin/pre-albumin position and LDL remained at the origin (150). The lipoprotein electrophoretic pattern given by the serum of the mouse (*M. musculus*) did however resemble that of the rat in that both were dominated by HDL (150). Kirkeby (152) also reported HDL (concentration 206 mg/dl) to be a major component in mice, although LDL levels were high (189 mg/dl) and generally higher than those (29 and 13 mg/dl of LDL and VLDL, respectively) detected by Mills and Taylaur (6).

Unfortunately, the only other investigation of murine (blood) plasma lipoproteins was performed on material from ascites tumor-bearing animals by Mathur and Spector (153), and an effect of the peritoneal tumor cells on the plasma lipoprotein profile in these animals cannot be discounted. The latter authors (153) isolated the total plasma lipoproteins by ultracentrifugation at a density of 1.21 g/ml, and subsequently prepared VLDL, LDL, and HDL by agarose column chromatography. Quantitative measurements were not made, but if protein contents of 10, 20, and 50% are assumed for mouse VLDL, LDL, and HDL, respectively, then consideration of the elution profile based on protein content would suggest that murine plasma contains similar levels of LDL and HDL, with lesser concentrations of VLDL. Mouse LDL appeared particularly heterogeneous in size (and density) as judged by a broad elution peak. Polyacrylamide gel electrophoresis in SDS of the delipidated apoproteins indicated that the major component of VLDL and LDL had a high molecular weight (>100,000) and thus was possibly an analogue of apolipoprotein B; the principal HDL apolipoprotein had a molecular weight of 27,000, corresponding to apo A-I in man. Small amounts of peptides less than 12,500 in size were detected in all three mouse lipoprotein classes and may represent analogues to the human C apolipoproteins.

The paucity of data on mouse lipoproteins, evident from the above discussion, is particularly surprising in view of the many mutant forms of this species; a number of these involve major perturbations of lipid metabolism and might provide interesting models for experimental studies of disorders common to man.

The anomalies described by several investigators in the distribution of low and high density lipoprotein species in the rat have been noted by Calvert (140), who has summarized the rather exceptional density intervals employed for isolation of LDL by ultracentrifugal flotation. While there are almost certainly significant differences in the qualitative and quantitative aspects of the rat serum lipoprotein profile due to strain (e.g., Wistar, Sprague-Dawley, and Osborne-Mendel (6, 140)), it would appear that a mutual and marked overlap of low (apo-B-containing) and high (apo-A-containing) density particle species is common to them; only Camejo (154), has reported a satisfactory separation of LDL from HDL at a density of 1.063 g/ml in adult Sprague-Dawley rats, and the weight of current evidence does not concur with this observation.

Comparison of the available and rather meager data in the literature on the distribution of serum lipoproteins in the rat (**Table 14**) reveals the disturbing finding that large variations in lipoprotein levels have been found in animals of the same strain. These discrepancies are difficult to explain entirely on the basis of nutritional status, and serve to remind investigators of a problem inherent to the pooling of small blood samples from several animals. For example, in fed, male Sprague-Dawley animals, a twofold difference in VLDL and threefold ranges in both LDL and HDL levels are apparent. HDL is the major class in all cases and tends to fall, with few exceptions, into the range of 100–250 mg/dl serum. Extended fasting (24 hr in the case of the Wistar rats studied by Calandra et al. (155)) apparently leads to diminished VLDL and LDL concentrations, although the 16hr-fasted Holtzman rats of Narayan et al. (159) maintained relatively high concentrations of both these lipoproteins. A differing susceptibility of individual strains to fasting cannot therefore be excluded. The data are insufficient to permit comment on any association between profile and sex. On the contrary, age is a major factor in determining circulating lipoprotein levels, as illustrated by the data of Schonfeld, Felski, and Howald (158) in Zucker lean control rats of different ages; such findings have been confirmed more recently by Malhotra and Kritchevsky (160) in animals of the Wistar strain, although the latter authors omitted analysis of lipoprotein protein.

The chemical compositions reported for rat lipoproteins are reviewed in **Table 15;** data lacking quantitation of cholesteryl ester have been excluded. The compositions of the VLDL fractions isolated by sequential ultracentrifugation were uniformly higher in triglyceride content (61–75%) than those separated by the rate zonal procedure (49.6%), although the

TABLE 14. The serum lipoprotein distribution in the rat[a]

Strain	Nutritional Status	Sex	VLDL	LDL	HDL	Methodology
	fasted			170[b]	313	Paper electrophoresis (152)
	fed		present	41	140	Analytical ultracentrifugation (116)
	fed		not determined	15–25	108–193	Analytical ultracentrifugation (149)
Wistar			66	16	246	Analytical ultracentrifugation (6)
Wistar	fasted	female	36	18	112	Chemical analysis (155)[c]
Sprague-Dawley	fed	male	107	56	240	Chemical analysis (156)[c]
Sprague-Dawley	fed	male	46	16	80	Chemical analysis (157)[cd]
Zucker lean	fasted	adult	87	62	241	Chemical analysis (158)[c]
		young	42	23	123	
Holtzman[e]	fasted	male	70	50[f]	216	Chemical analysis (159)[cd]

[a] Concentrations are expressed as mg lipoprotein/dl serum.
[b] Includes VLDL.
[c] Data based on lipoprotein recoveries after ultracentrifugal flotation (involving losses).
[d] Cholesterol as total sterol, thus lacking correction for cholesteryl ester (fatty acid) content.
[e] Maintained on a semisynthetic diet.
[f] Inclusive of HDL_1 concentration.

Density limits employed for analytical ultracentrifugal analyses were VLDL < 1.006, LDL 1.006–1.063, and HDL, 1.063–1.21 g/ml. In chemical analyses, LDL and HDL in young Zucker leans are of d 1.006–1.070 and 1.070–1.21 g/ml, respectively (158), and of d 1.006–1.063 and 1.063–1.21 g/ml, respectively, in adults of the same strain; in Wistar rats (155), LDL and HDL are of d 1.006–1.050 and 1.050–1.21 g/ml, respectively, and of d 1.006–1.055 and 1.07–1.21 g/ml in Sprague-Dawley rats (157).

total neutral lipid content of the latter fraction (67%) was within the overall range (65–80%). Wide ranges were seen in the proportions of cholesteryl ester (1.9–17.0%), free cholesterol (2.6–8.9%), and phospholipid (9.6–18.3%); the rate zonal fraction displayed the highest contents of both cholesteryl ester and phospholipid. The ratio of esterified:free cholesterol varied widely, and several VLDL fractions showed inverse ratios of these lipids, i.e., values of EC/UC less than unity.

Inasmuch as the majority of investigators have not presented data on the physical properties, and especially particle size, of their VLDL fractions, little further interpretation of the above results is possible. However, by chemical analysis of a series of VLDL fractions, each of whose size distribution was determined, Mjøs et al. (166) were able to examine the relationship between particle diameter and the percentage of particle volume occupied by surface components (protein, phospholipid, and free cholesterol). This relationship was found to be essentially the same in rat as in human VLDL (73), indicating that rat particles consist of an apolar core and surface (polar) monolayer of 21.5 Å thickness. Rat VLDL therefore appears subject to the same structural constraints as human VLDL.

The heterogeneous nature of rat LDL has been described by several authors on the basis of analytical ultracentrifugal analysis (6, 116, 154), and more recently as a result of the application of an electrophoretic technique of high resolution (163). It is evident, therefore, that chemical analysis of rat LDL isolated in a wide density interval, such as 1.006–1.063 g/ml, is of little significance. Comparison of such a fraction (Table 15) with analyses either of IDL (d 1.006–1.019 or 1.006–1.020 g/ml) or of LDL of d 1.019–1.063 or 1.02–1.05 g/ml serves to illustrate this point, and at the same time reveals the degree of heterogeneity and complexity of molecular species present in the "classical" density fraction. Wide ranges in the proportions of all LDL constituents occur; particularly noteworthy are cholesteryl ester, which varies from 12.5% in IDL to ca. 40% in a fraction of d 1.019–1.063 g/ml, and triglyceride, which is maximal in IDL (46.2%) and minimal (5.5%) in a 1.019–1.063 g/ml fraction. Free cholesterol also showed a wide range (4.8–19.1%), giving rise to ratios of esterified:free cholesterol from about 0.5 to 5. Only limited analyses of the physical properties of these preparations were performed (161), but at least by electron microscopic examination, rat IDL was highly heterogeneous, containing at least three particle species. By contrast, LDL of d 1.019–1.063 g/ml was homogeneous in size (mean diameter 200 Å) and shape; some indication of subunit structure was detectable (161).

Rat LDL prepared by rate zonal centrifugation displayed the lowest protein content (12.6%) of all the fractions examined, though apart from this disparity, it could not be distinguished from material isolated by conventional ultracentrifugation. Whether the distinct compositions of the VLDL and LDL described by Dunn, Wilcox, and Heimberg (164) are the result of rate zonal procedure, or whether they

TABLE 15. Chemical composition of rat serum lipoproteins

Density limit (g/ml)[d] (Reference)	VLDL								IDL	
	<1.006 (6)	<1.006 (165)	<1.006 (166)	<1.006 (156)	<1.006 (161)	<1.006[c] (162)	<1.006 (155)	[a]	1.006–1.019 (161)	1.006–1.020 (151)
	mean % (weight)								*mean % (weight)*	
Cholesteryl ester	4.3	5.6	1.9	4.9	4.2	2.6	8.9	17.0	12.5	14.1
Free cholesterol	4.0	2.8	2.7	3.9	8.9	2.6	4.3	5.2	18.1	9.4
Triglyceride	75.0	70.1	73.6	68.1	61.1	74.2	61.0	49.6	41.1	46.2
Phospholipid	11.2	11.2	12.6	13.6	12.0	9.6	13.8	18.3	12.0	11.4
Protein	5.4	10.3	9.4	9.5	13.8	11.0	12.0	10.0	16.3	18.9

[a] Isolated by zonal ultracentrifugation; hydrated density not determined; data calculated from ref. 164.
[b] Isolated by Geon-Pevikon block electrophoresis from a mixture of LDL and HDL_1 of d 1.02–1.063 g/ml; data calculated from ref. 163.
[c] Calculated from ref. 162.
[d] Fractions isolated by repetitive sequential ultracentrifugation unless otherwise stated.

are coincidentally linked to differences in strain, age, and nutritional state, remains unknown, although Hill and Dvornik (167) have cited evidence for an effect of strain, age, and body weight on the composition of the major rat lipoproteins and especially on that of VLDL.

Electrophoresis on Pevikon block of LDL of d 1.02–1.063 g/ml facilitated the isolation of rat HDL_1 by Weisgraber, Mahley, and Assmann (163); this particle would therefore normally be a constituent of rat LDL as conventionally isolated and indeed, may account for up to half of the total cholesterol transported in the density range 1.02–1.063 g/ml in this rodent. HDL_1 was enriched in cholesteryl ester (Table 15) and essentially indistinguishable in composition from (LDL) fractions of density 1.019–1.063 g/ml; it displayed α_2-mobility and a mean particle size of 192 Å (range 140–290 Å), thereby resembling the corresponding fractions of d 1.04–1.06 g/ml from man and the cow and seal (mean diameters 140–160 Å) (168).

Rat HDL of d 1.063–1.21 g/ml displayed the high protein content typical of other mammals (6); it was also deficient in triglyceride. Twofold and fourfold ranges are evident in the proportions of cholesteryl ester and free cholesterol, respectively, in this density fraction, but such variance almost certainly arises from dissimilarities in the overall particle contents of individual preparations. Thus, the HDL_2 subfraction (d 1.05–1.125 or 1.063–1.125 g/ml) tended to be distinct from HDL_3 (d 1.125–1.21 g/ml), although the only consistent difference in the data summarized in Table 15 is an increased protein content in HDL_3, thus agreeing with data in man (4, 137). Rat HDL_2 is comprised of essentially spherical particles with a mean diameter of 136 Å (161), while HDL_3 is more variable in shape and most particles are of 50–60 Å in size (161). The subfraction of d 1.075–1.175 g/ml is intermediate in size (mean diameter 114 Å) (165) between HDL_2 and HDL_3.

Separation and quantitation of the major phospholipid classes, and of their constituent molecular species, has been performed in the major classes of rat lipoproteins (including chylomicrons) by Kuksis and colleagues (162, 169) and by Malhotra and Kritchevsky (160), although the former authors analyzed VLDL and LDL phospholipids as a single fraction. In both young and old rats, phosphatidylcholine was the predominant phospholipid in all lipoprotein classes (VLDL, IDL, LDL of d 1.019–1.063 g/ml, and HDL), representing at least 60% of the total. Sphingomyelin generally accounted for a smaller proportion of the total in each class than it did in the corresponding human lipoproteins (44), although all the lipoprotein classes in old rats contained more sphingomyelin and less lecithin than those of young animals.

In HDL of normal rats, the major molecular species of phosphatidylcholine were 34:2, 36:2, and 38:4 in carbon number, corresponding to the fatty acids 16:0 and 18:2, 18:0 and 18:2, and 18:0 and 20:4, respectively (162). By contrast, the predominant species of sphingomyelin in HDL contained 42 carbon atoms, accounting for some 65% of the total. The cholesteryl ester fatty acids of rat VLDL and LDL can be readily distinguished from those in the human lipoproteins by their elevated levels of arachidonate and diminished linoleate content (156, 164, 169). Some discordance is however apparent upon comparison of reported values for arachidonate in rat HDL; this acid accounted for some 65% and 40% of the fractions isolated by Dunn et al. (164) and by Mookerjea, Park, and Kuksis (169) respectively, but to less than 10% in the analysis of de Pury and Collins (156). All HDL fractions did contain less linoleate (range 18–30%)

LDL						HDL									
1.006–1.063 (6)	1.006–1.063 (156)	1.02–1.05 (155)	1.019–1.063 (161)	1.019–[c] 1.063 (162)	[a]	HDL_1[b] (163)	1.063–1.21 (6)	1.063–1.21 (156)	1.063–1.21 (161)	1.05–1.125 (155)	1.063–[c] 1.125 (162)	1.125–[c] 1.21 (162)	1.125–1.21 (155)	1.075–1.175 (165)	[a] (164)
		mean % (weight)													
26.8	26.2	12.8	15.0	39.8	37.8	36.8	33.6	17.6	15.0	19.4	27.3	25.9	10.5	23.6	26.0
8.6	12.8	17.7	19.1	4.8	11.1	11.9	3.6	5.7	14.8	6.1	3.1	2.7	9.8	5.1	4.5
18.5	14.3	31.8	12.2	5.5	9.9	3.4	1.7	0.6	2.8	7.5	1.8	3.2	7.6	1.2	0.6
21.2	18.7	19.2	27.2	27.4	28.7	22.2	28.1	15.1	26.0	28.2	29.8	26.4	21.1	25.9	29.3
24.9	28.0	18.5	26.5	22.0	12.6	25.7	32.8	61.0	41.4	39.5	38.0	46.0	51.0	44.3	39.5

than the corresponding human fractions (some 55% (65)).

Triglyceride fatty acid patterns in the VLDL, LDL, and HDL of rat and human serum were alike (65, 156, 164, 169), oleate predominating (ca. 30–40%), with lesser amounts of linoleate (20–30%) and palmitate (15–35%); arachidonate levels were rather higher in the rat triglycerides, and particularly in the LDL class. Little distinction could be made between the phospholipid fatty acids of the rodent and human lipoproteins (65, 154, 164, 169), although the rather wide range seen in reported values for the rat phospholipids is notable. Particularly high arachidonate levels (21–24%) were detected in the rat fractions analyzed by Dunn et al. (164).

These data indicate a central role of rat lipoprotein cholesteryl esters in the transport of essential fatty acids, especially of arachidonate. Moreover, they suggest either some difference between the substrate specificity of lecithin:cholesterol acyl transferase activity (4, 94, 170) in rat and man, or alternatively, that lipoprotein cholesteryl esters have differing origins in the two species. As in man, the role of HDL in lipid transport (particularly of cholesterol) in the rat remains to be established. It would however seem that these macromolecules, together with LDL, are important vehicles for transport of dietary sterols, e.g., of β-sitosterol and campesterol (171). Such a function is clearly not unique to mammalian lipoproteins, since, as already noted, the high density substances of invertebrates also fulfil this role, while the intense brownish color of HDL in trout suggests their involvement in pigment/exogenous sterol transport in this marine vertebrate.

The complexity of the apolipoproteins in the rat was first evident from the early studies of Koga, Bolis, and Scanu (172), Bersot et al. (173), and Schonfeld et al. (158), and indeed their data were conflicting on several points, which have been discussed by Calvert (140). These results did however indicate the presence of a single major apolipoprotein of high molecular weight in LDL (>150,000 as judged by its exclusion from Sephadex G-150), which represented ca. 25% of apo VLDL, and which was presumed to be an analogue of human apo B. Some support for this contention arises from the similarity in carbohydrate composition of rat and human LDL (174). Surprisingly, rat apo B has subsequently received little attention, and even its amino acid composition is lacking in the literature. Its presence has often been concluded from the failure of part of an apoprotein (e.g., apo VLDL), to migrate into polyacrylamide gels of high (>5%) monomer concentration, and more exacting criteria for its identification are desirable.

The low and intermediate molecular weight apolipoproteins (<20,000 and 20,000–50,000, respectively) of rat plasma have subsequently received a good deal of attention. In pioneering studies, Swaney, Reese, and Eder (175) electrophoresed apo HDL in SDS-polyacrylamide gels, thereby permitting a direct comparison between the rat and human (apolipoprotein) band patterns. The nomenclature developed for the human apolipoproteins was used for the rat apoproteins. These studies largely agreed with those reported earlier (172, 173) revealing three major polypeptides in rat HDL; the major constituent (50–60%) had a molecular weight of 27,000 and was comparable to human apo A-I; another (10–15% of total) of molecular weight 46,000 was termed A-IV and was recently found in human VLDL (176), and a third (10–15%) of molecular weight 35,000, rich in arginine, was termed the arginine-rich protein. The remaining 15–20% of rat apo-HDL consisted of a group of low molecular weight (8–12,000) "C" peptides. These investigations provided the first indication that rat HDL differed markedly from that of man

(4) in possessing substantial amounts of apo E, i.e., the arginine-rich protein.

Employing a combination of gel filtration and ion-exchange chromatography, Herbert and collaborators (177) characterized the low molecular weight proteins of rat HDL noted above. These consisted of five proteins, representing homologues of the human A-II, C-I, C-II, and C-III apolipoproteins. The A-II protein, although existing in monomeric form, closely resembled human A-II in amino acid composition and in its blocked NH_2-terminal residue. A striking similarity was also noted by Herbert et al. (177) in the quantitative and qualitative features of the rat and human C apolipoproteins. The C-I peptides of the two species were alike in amino acid composition, as were their C-II components, and these apolipoproteins uniformly lacked carbohydrate; rat apo C-II may activate lipoprotein lipase (177). Rat apo C-III could be resolved into two polymorphic forms, C-III-0 and C-III-3, as a result of its variable carbohydrate content. Thus C-III-0 lacked sugar residues whereas C-III-3 contained one galactosamine and three sialic acid residues per mole. While such polymorphism is also seen in human C-III, apo C-III-0 is usually a minor form, and apo C-III-I and C-III-2 predominate (4, 178). Some small dissimilarities between human and rat C-III were also apparent in their amino acid compositions, and mainly concerned their glycine, alanine, leucine, histidine, and isoleucine contents.

These findings have recently been extended by Swaney and Gidez (179) who employed isoelectric focusing to further fractionate the rat low molecular weight apolipoproteins. The A-II, C-II, C-III-0, and C-III-3 components focused as single bands with isoelectric points of 4.83, 4.74, 4.67, and 4.50, respectively; electrophoresis in SDS-gel slabs suggested that these apolipoproteins had molecular weights of 8,000, 8,000, 10,000, and 11,000, respectively. While a molecular weight of 7,000 was established for rat apo C-I, the authors failed to achieve a satisfactory resolution of this basic peptide by isoelectric focusing, even in a high (7–9) pH range.

Two of the major apolipoproteins of rat HDL, i.e., A-I and A-IV, were originally purified by Swaney et al. (139), while a third, the arginine-rich protein (ARP; (163)) or apo E has been characterized both by the latter workers and by Weisgraber et al. (163). After an initial separation from apo HDL by gel filtration chromatography followed by preparative SDS-polyacrylamide gel electrophoresis (139), rat apo A-I showed an overall similarity in amino acid profile to its human counterpart, although it possessed isoleucine which is absent in man (3, 4); the two proteins probably possess the same N-terminal amino acid (aspartate). Rat A-I could be resolved into four to five bands upon isoelectric focusing with pI of 5.55 to 5.82. The A-IV protein was immunologically non-identical to the other HDL polypeptides, and had a molecular weight of 46,000 and a distinctive amino acid composition. Gidez, Swaney, and Murnane (180) found by isoelectric focusing that this apolipoprotein was polymorphic, banding in four to six positions in the pH range 5.46 to 5.82 (180), but overlapping to a significant degree with bands of apo E.

Rat apo E, a polypeptide common to rat VLDL and HDL, has a molecular weight (35,000) and amino acid composition (163, 139) similar to the human polypeptide isolated by Shore and Shore (181). Indeed, this apolipoprotein which is apparently implicated in nascent HDL formation and secretion from the liver (165), is present in homologous forms in the dog (182), rabbit (183), pig (184), and monkey (185). Again, it exhibits polymorphism, at least as judged by its behavior upon isoelectric focusing; it is resolved as four to six bands in the pH range 5.31–5.46 (180). Such polymorphism may arise from its variable content of sialic acid (163).

Some information is available on the molecular species into which the serum apolipoproteins are organized in rat HDL, and this knowledge is of particular relevance to lipoprotein classification and nomenclature, since it remains to be seen whether the lipoprotein family hypothesis propounded by Alaupovic and colleagues (7) for the human system is equally applicable to animal species. It may also have special bearing on our understanding of HDL metabolism. Swaney et al. (139) have calculated a molar ratio of A-I:Apo E:A-IV in normal rat HDL of 3.7:1.0:0.96. Since these values approach whole numbers, the latter workers interpret their data as favoring a homogeneous HDL population.

Heparin affinity chromatography provides an experimental approach to the problem of HDL heterogeneity, particularly since apo E binds selectively to this polyanion. Using this approach, Quarfordt et al. (186) have described the isolation of a major subfraction of rat HDL containing apo A-I and virtually no apo E, and a minor fraction (10% of total HDL) possessing essentially only apo E in its protein moiety. These two species differed markedly in lipid composition, but were of similar size, and ultracentrifugal and electrophoretic behavior. Such observations are consistent with the lipoprotein family hypothesis (7).

Apolipoprotein levels are becoming of increasing importance in experimental studies in animals, and it is therefore of interest to note that a number of workers have quantified certain of the serum apo-

lipoproteins in normal rats. The most comprehensive study to date has been that of Bar-On, Roheim, and Eder (187), in which proportions of apolipoproteins B, C, A-I, A-IV and E in VLDL, LDL, HDL, and the fraction of density greater than 1.21 g/ml (d > 1.21) were described. Apo B was principally present in VLDL, whereas the major portions (50% of total) of the other apolipoproteins were present in HDL. Rather higher proportions of both apo E and apo A-IV (36% and 31% of the serum level, respectively) have subsequently been found in the bottom (d > 1.21) fraction however (188); these results may be partially explained by apolipoprotein losses during ultracentrifugation, as indicated by Mahley and Holcombe's (13) experiments. This suggestion has been confirmed by the findings of Wong and Rubinstein (189) who detected 40–50% of apo E in the bottom fraction after ultracentrifugation. Total serum levels of apo E vary from 18 to 31 mg/dl according to the method, strain of rat, and possibly nutritional status (13, 189, 190). Little information is available on apolipoprotein C levels, although Kim and Kalkhoff (191) have reported quantitation of C-II and C-III species in rat VLDL and HDL based on densitometric scanning of stained urea-polyacrylamide gels.

The guinea pig (*Cavia porcellus*) is distinct from the rat in its ready and rapid response to dietary lipid, especially cholesterol. Moreover its serum lipoprotein profile under conditions of a normal, low-fat diet is quite different from that of *R. norvegicus* (5, 140), in that low-density, apo B-containing particles represent at least 80% of the total lipoproteins of d < 1.21 g/ml (90, 192, 193). The two groups who have reported observations on guinea pig lipoproteins concur that substances with the characteristics of low density, apo B-containing particles extend to the region of 1.09–1.100 g/ml (90, 192–194). Furthermore, as noted earlier, HDL levels are extremely low in Cavia and this has limited their study. However, the fraction of d 1.09–1.21 g/ml, though shown to contain small amounts of LDL by electron microscopy, has a chemical composition not unlike that of human HDL (i.e., 39% protein, 25% phospholipid, 9% free cholesterol, 25% cholesteryl ester, and 2% triglyceride) (192). The chemical and physical properties of LDL in normal guinea pigs have been summarized (140). It is noteworthy that recent studies of LDL of d 1.007–1.100 g/ml show, as might be expected, that their diameters (mean 214 Å, (90)) are smaller than those of LDL of d 1.007–1.063 g/ml (193). Moreover, the former fraction was shown to be highly homogeneous and comparable in morphology to that seen in man (86).

Some variability is apparent in reported analyses of guinea pig VLDL (90, 192, 193); these dissimilarities concern primarily the proportions of esterified and free cholesterol, and presumably are as a result of diet. Although both Chapman and Mills (90) and Sardet, Hansma, and Ostwald (193) examined VLDL from overnight-fasted animals, the VLDL of the former investigators showed a considerably narrower particle range (300–800 Å; mean diameter 463 Å;) than that of the latter workers (range 400–1300 Å). This observation is entirely consistent with the higher triacylglycerol content (72%) of the VLDL found by Sardet et al. (193). The subfractionation of guinea pig VLDL by gel filtration chromatography has been described by Barter, Faergeman, and Havel (195), who noted that, unlike the rat, the cholesteryl ester content of these particles did not increase with diminution in particle size.

Little information has been reported on the fatty acid compositions of lipoprotein lipids from guinea pigs. Chapman, Mills, and Taylaur (196) found linoleate to be the major fatty acid of LDL (d 1.006–1.063 g/ml) cholesteryl esters (61%) but a minor component of these esters in VLDL (13.2%). By contrast, linoleate represented in excess of 70% of the cholesteryl esters of both VLDL and LDL (d 1.006–1.063 g/ml) in the study of Barter et al. (195). The phospholipid fatty acid patterns of VLDL and LDL were essentially identical (196), the major components being stearate and linoleate. While linoleate was also a dominant fatty acid in VLDL and LDL triglycerides (196), their fatty acid patterns were distinguished by the twofold higher proportions of stearate and oleate in the latter. The rather low levels of arachidonate in the three lipid esters, and particularly in cholesteryl esters (195, 196), reveal a marked contrast between rat and guinea pig in the mode of transport of essential fatty acids.

Considerable progress has recently been made in our knowledge of guinea pig serum apolipoproteins (90, 194, 197, 198). The earliest investigation was that of Chapman, Mills, and Ledford (197), who fractionated the high and low molecular weight apolipoproteins of VLDL, LDL, and HDL, and partially characterized them. The major component of apo VLDL and apo LDL, accounting for some 40–70% and 70–90% of the total apoprotein, respectively, was a high molecular weight apoprotein which eluted in the void volume following gel filtration chromatography in SDS-containing buffer and which was insoluble in tetramethylurea. Its molecular weight in SDS-polyacrylamide gel (>250,000), solubility properties, and amino acid composition suggested that this protein was analogous to human apo B, a finding consistent with the cross-reactivity of guinea pig LDL with antiserum to human LDL (127, 192).

Using an essentially identical approach, Guo et al. (194) have confirmed the presence of an apo B-like protein in the LDL of control guinea pigs.

The major protein components of HDL (1.100–1.121 g/ml) were resolved as two bands of low mobility upon electrophoresis in urea-polyacrylamide gel (197); one of these, band VI, was found to resemble human apo A-I in amino acid composition following its elution from the gel and subsequent hydrolysis. The principal differences between the human and guinea pig polypeptides were the presence of isoleucine in guinea pig A-I, and its apparent lack of methionine and proline; the predominant amino acids in the A-I of both species were glutamic and aspartic acids, leucine, alanine, and lysine. Guo et al. (194) isolated the major protein of control HDL (d 1.09–1.20 g/ml) by elution from SDS-polyacrylamide gel slices. Although the molecular weight of this polypeptide was estimated as 25,000, thereby resembling human A-I (3, 4) its amino acid composition was judged strikingly different from both the human and rat apolipoproteins. Comparison shows, however, that apo A-I isolated by Guo et al. (194) is no less unlike its human counterpart than the polypeptide (band VI) identified earlier by Chapman et al. (197). Indeed, the A-I protein of the former workers had a four-fold greater content of glycine than human A-I, and a three-fold higher content than the band VI protein. Its glutamic acid content (13.1%) also differed markedly from that of band VI (21.4%) and human A-I (18.3%) (197). Reasons for such dissimilarities are not apparent, although the methodology used by Chapman et al. (197) did not ensure the purity of the band VI component. We may, however, conclude that an apolipoprotein analogous to human A-I is present in Cavia, and it will be of interest to determine whether its distinct amino acid composition is related to differences in its function or ability to stabilize the lipid complement of HDL.

The second major apolipoprotein identified in urea-polyacrylamide gels of guinea pig apo HDL, band VI-B in the nomenclature of Chapman et al. (197), migrated to a position resembling that of human apo A-II. Moreover, it shared high contents of lysine, glutamic acid, and leucine with the human protein. Any further identification of this component must however await its isolation and characterization. A number of additional apolipoproteins could be resolved upon electrophoresis in basic urea-polyacrylamide gel of the tetramethylurea-soluble apolipoproteins of both guinea pig VLDL and LDL. Two of these, bands IX and X, were rich in arginine and of similar electrophoretic mobility to the arginine-rich proteins (apo E) of man (181) and rabbit (183). The guinea pig proteins tended to be distinct in amino acid composition however, and particularly in their elevated aspartate and diminished glutamate contents.

Using chromatographic techniques and the larger amounts of apo HDL obtainable in cholesterol-fed guinea pigs, Guo et al. (194) have isolated an arginine-rich protein which appears homologous to that of man and rat with only minor differences in amino acid composition. The molecular weight of the guinea pig protein was 34,000. A number of other low molecular weight apolipoproteins are detectable in the protein moieties of guinea pig VLDL and LDL. One group (bands II–IV (197)) superficially resembles the human C proteins in its high electrophoretic mobility; these peptides are quite distinct from the latter in amino acid composition however. An additional component, denoted Band V (197), was consistently resolved in apo VLDL and apo LDL, but on the basis of amino acid composition, it did not appear to possess a human counterpart. This contention has recently been confirmed by Meng, Guo, and Ostwald (198), who isolated a polypeptide of molecular weight 34,000 from control guinea pig LDL, and showed that it comigrated with apo E on SDS-polyacrylamide gel. Its amino acid composition resembled that of the band V component, and 34% of the total weight was carbohydrate (198).

The apolipoprotein complement of guinea pig lipoproteins is clearly distinct from that of rat and man, and further work is needed to evaluate the roles of the various peptides. The guinea pig may also differ from man and other animals in the characteristics of its lipolytic system, since it appears to lack a peptide analogous to the human and rat C-II activator proteins. Nonetheless, it would appear to possess an efficient lipid clearing system, since Cavia is particularly resistant to the development of a hypertriglyceridemia, even when fed diets high in fat (90, 192).

Carnivores

The order Carnivora comprises a number of families, among which members of the Canidae (e.g., the dog, *Canis familiaris*; the dingo, *Canis dingo*), the Felidae (e.g., the cat, *Felis domesticus*; the lion, *Panthera leo*) and the Mustelidae (e.g., the ferret, *Mustela putorius furo* L.; the mink, *Mustela vison*) have been the subject of lipoprotein studies. In the vast majority of Carnivores, data in the literature extend only to serum lipid analyses and in some cases, to lipoprotein concentrations; **Table 16** summarizes current information. At this juncture, it is noteworthy that pinnipeds, such as the harbor seal (*Phoca vitulina*) and California sea lion (*Zalophus californianus*) are Carnivores, but since they have often been studied

TABLE 16. The serum lipoprotein distribution in members of the Order Carnivora

Species	VLDL	LDL	HDL_1	HDL	Methodology
		mg/dl			
Canidae					
Dog (*Canis familiaris*)		183[a]		381	Paper electrophoresis (152)
Dog (*Canis familiaris*)	n.d.	32 (25–38)		576 (332–996)	Analytical ultracentrifuge (149)
Dog (*Canis familiaris*)	15	26	13[c]	343	Chemical analysis (142)[b]
Dog (*Canis familiaris*)	26 (4–107)	26 (17–120)		113 (359–812)	Cellulose acetate electrophoresis (199)
Dog (*Canis familiaris*)	73	135		479	Chemical analysis (200)[b]
Dingo (*Canis dingo*)	5	99		n.m.	Analytical ultracentrifuge (6)
Felidae					
Cat (*Felis domesticus*)		114[a]		359	Paper electrophoresis (152)
Cat (*Felis domesticus*)	n.d.	70 (34–108)		267 (94–346)	Analytical ultracentrifuge (149)
Jaguar (*Panthera onca*)	n.d.	286		n.m.	Analytical ultracentrifuge (6)
Lion (*Panthera leo*)	2	156		n.m.	Analytical ultracentrifuge (6)
Lynx (*Lynx lynx*)	n.d.	59		n.m.	Analytical ultracentrifuge (6)
Mustelidae					
Ferret (*Meles putorius furo* L.)	37	112		548	Chemical analysis (201)
Badger (*Meles meles*)[d]	60	250–1200		500–900	Analytical ultracentrifuge
Red fox (*Vulpes vulpes* L.)[d]	15–30	150–180		600–800	Analytical ultracentrifuge

[a] Includes VLDL as β-lipoprotein.
[b] Cholesterol as total sterol; no correction for cholesteryl ester content.
[c] Isolated from LDL by Pevikon block electrophoresis.
[d] Dr. P. M. Laplaud, unpublished observations.
n.d., not detectable; n.m., not measured. Density limits correspond to VLDL d < 1.006, LDL 1.006–1.063, and HDL 1.063–1.21 g/ml unless otherwise noted.

in association with other marine mammals, notably whales and dolphins, they will be considered together with the latter, i.e., the Cetaceans.

As previously noted (140), HDL is the major lipoprotein class in the dog, and the levels are generally greater than those typical of man (65). VLDL concentrations are particularly low, with LDL ranging from 25 to 180 mg/dl serum. HDL_1, apparently spread over a wide density interval (1.025–1.100 g/ml) is present in minor amounts. It is noteworthy that while Mahley and Weisgraber (142) reported a total serum lipid concentration of 465 mg/dl in the plasma of foxhounds, the lipid content of the isolated lipoproteins (VLDL, LDL, HDL_1, and HDL_2) only accounted for 241 mg/dl. The question therefore arises as to whether the dog transports large amounts of lipid in VHDL of d > 1.21 g/ml. Insufficient data are available to permit comment on any possible relevance of breed to lipoprotein profile in the dog. On average, LDL levels in the various cats (Felidae) examined to date tend to be greater than those in Canis sp., although they appear to lack VLDL; HDL is preponderant in the domestic cat (*F. domesticus*). The lipoprotein profile in Mustelidae (ferret, badger and red fox; Table 16) is typically dominated by HDL whose concentrations are at least twice those of man (65). A notable exception to this is the badger (*Meles meles* L.), which is seasonally hypercholesterolemic (290). Thus its plasma lipoprotein levels vary according to season, LDL reaching a maximum during the cold months of the year. This species, as well as other hibernators such as the hedgehog, should prove an invaluable model for study of the relationship of locomotor activity and endocrine function to the serum lipoprotein spectrum.

Chemical analyses have been described for the lipoproteins of only two members of the order Carnivora, the dog (140) and ferret (201); unfortunately data on cholesteryl ester content have generally been omitted. Canine VLDL appear variable in composition, especially in the proportion of cholesteryl esters (**Table 17**), a similar comment being applicable to LDL. The LDL_1 subfractions separated by zonal ultracentrifugation ("LDL_1", (204)) and by flotation in the interval 1.006–1.019 g/ml (202) are of contrasting composition. Thus, the elevated triglyceride (77%) content of the fraction described by Blomhoff, Holme, and Ostrem (202) is more akin to VLDL than LDL. LDL_2 of d 1.019–1.063 g/ml was poor in cholesteryl ester and rich in triglyceride (202). However, purification of this fraction by electrophoresis (142) yielded a lipoprotein with β-mobility relatively rich in cholesteryl esters (24.5%); the major fatty acid was oleate (205). The major protein component of canine LDL was later found to resemble human apo B; this was confirmed by the cross-reactivity of canine LDL with an antiserum to the human protein (204). Phosphatidylcholine was the major LDL phospholipid species (205).

HDL_1, similarly purified from a d 1.006–1.063 g/ml

TABLE 17. Chemical composition of serum lipoproteins in the dog (weight %)

	VLDL			LDL			HDL_1[b]	HDL		
Density limit (g/ml)[a]	<1.006	<1.006[b]	1.006–1.019	"LDL_1"[c]	1.019–1.063	1.006–1.063[b]		1.063–1.21	1.063–1.21	1.087–1.21[b]
Reference	(202)	(142)	(202)	(204)	(202)	(142)	(142)	(202)	(203)	(142)
Component										
Cholesteryl ester	2	18.2	3	21.5	11	24.5	36.5	15	24.7	22.8
Free cholesterol	6	3.0	2	6.5	11	5.2	7.7	6	4.6	4.9
Triglyceride	68	54.5	77	18.0	30	26.6	1.9	2	0.7	0.6
Phospholipid	10	15.2	6	29.5	20	22.4	34.6	42	27.7	33.0
Protein	14	9.1	12	24.5	28	21.3	19.2	35	41.5	38.7

[a] Fractions were isolated by sequential ultracentrifugation unless otherwise stated.
[b] Purified by Pevikon block electrophoresis; data calculated from ref. 142.
[c] Isolated by zonal ultracentrifugation (204); hydrated density not determined.

fraction, was also enriched in cholesteryl ester (142), and contained canine A-I as its major apolipoprotein (142). This lipoprotein had a high phospholipid content (34.6%). Canine HDL_1 could be further fractionated into several subpopulations by precipitation with heparin/manganese (144), varying principally in protein and cholesterol content. The subfraction most susceptible to precipitation was rich in the arginine-rich apoprotein and competed effectively with LDL for binding to the LDL receptor on cultured fibroblasts. This apoprotein is particularly abundant in HDL_c, a lipoprotein whose formation in large amounts is induced by cholesterol feeding to hypothyroid dogs (144, 205, 207). Indeed, preparation of this lipoprotein in large amounts facilitated the purification of canine apo E, (182), whose molecular weight, mobility in urea-polyacrylamide gel, and amino acid composition showed it to be homologous with the arginine-rich proteins of a number of other species (147, 163, 181, 183, 184).

The denser HDL, termed HDL_2 by Mahley and Weisgraber (142), showed the high protein and phospholipid typical of mammalian species. Cholesteryl ester tended to represent a higher proportion of the canine than of human HDL (44, 137); its major fatty acid was linoleate (203). As in canine LDL, phosphatidylcholine accounted for the major portion of the phospholipid of the high density lipoproteins (203). Physicochemical studies of canine HDL (203) indicate its physical properties to be intermediate between those of human HDL_2 and HDL_3, a finding consistent with the presence of an unusually high degree of homogeneity in the canine lipoprotein (204). Furthermore, the protein moiety of this particle consists mainly of a 28,000 molecular weight polypeptide analogous to human apo A-I in amino acid composition (203). Sequence studies show only four residue changes in the first 33 amino acids at the NH_2-terminal end of canine apo A-I (206). Inasmuch as the canine protein does not immunologically cross-react with human apo A-I (206), and since these apolipoproteins exhibit differences in carboxyl-terminal sequence (203, 206), Nakai, Whayne, and Tang (206) proposed that the COOH-terminal end of apo A-I may play a central role as the antigenic site of the protein, whereas the NH_2-terminal end may play a common functional role. Some discordance in the carboxyl-terminal sequences of canine apo A-I reported by Edelstein et al. (203) (leu-ala-gln) and by Nakai et al. (206) (lys-leu-ala) is notable.

Recently, the presence of an apolipoprotein apparently homologous to rat and human apo A-IV has been described in canine (lymph) VLDL and plasma HDL_1 (208). The canine protein was of slightly lower molecular weight (44–45,000) than its rat and human counterparts (46,000) (139,176). Although the amino acid compositions of apo A-IV were alike in the three species, canine and human apo A-IV cross-reacted immunochemically in a reaction of partial identity, whereas the rat protein failed to react with an antiserum to canine apo A-IV (208). The chemical composition of the lipoproteins from a member of the family Felidae has not, to the author's knowledge, been described.

Members of the family Mustelidae have similarly been neglected. However, Cryer and Sawyerr (201) have compared the composition and apolipoprotein content of ferret lipoproteins with those of man. Their investigations suggest that ferret VLDL is poor in triglyceride and rich in phospholipid, while LDL is deficient in cholesterol and enriched in triglyceride. On the other hand, ferret and human HDL were alike. Insolubility in tetramethylurea suggested that major proportions of ferret apo VLDL (32.1%) and apo LDL (95%) consisted of a protein analogous to human apo B. Densitometric scans of the electrophoretic patterns of the tetramethylurea-soluble apolipoproteins of ferret VLDL, LDL, and HDL revealed an overall resemblance to their human counterparts. By virtue of its ability to activate triglyceride emul-

TABLE 18. Serum lipoprotein levels in the rabbit fed a low-fat, control diet[a]

Strain	Nutritional Status	VLDL[b]	IDL[b]	LDL[b]	HDL[b]	Methodology
Dutch belt	fed	25	85	105	140[d]	Analytical ultracentrifuge (215)
New Zealand White	fed	25	100	115	130[d]	Chemical analysis
		19	100	118	160	Analytical ultracentrifuge (6)
	fed		23		134	Analytical ultracentrifuge (212)
Fauve de Bourgogne	fasted	52	157		257	Gravimetric analysis (217)
	fed	present	121		102	Analytical ultracentrifuge (116)
	fed	n.d.	n.d.	58–62	70–124	Analytical ultracentrifuge (149)
			188[c]		40	Paper electrophoresis (152)

[a] Concentrations are expressed as mg lipoprotein/dl serum.
[b] VLDL, IDL, LDL, and HDL correspond to fractions of density < 1.006 g/ml (or S_f 20–400), 1.006–1.019 g/ml (or S_f 12–20), 1.019–1.063 (or S_f 0–12), and 1.063–1.21 g/ml, respectively, unless otherwise indicated.
[c] Includes VLDL as β-lipoprotein.
[d] HDL of d 1.081–1.21 g/ml.
n.d., not detectable.

sions for hydrolysis by lipoprotein lipase, ferret serum appears to contain a peptide analogue of rat and human apo C-II. Despite the lack of basic information on the distribution and chemistry of the lipoproteins of the mink (*Mustela vison*), Baranov and co-workers (209, 210) have employed this species for extensive investigations of the polymorphism of serum α_2-lipoprotein (d > 1.21 g/ml). More recently, the molecular distribution of allotypic determinants in mink VHDL has been documented (211).

Lagomorphs

The rabbit (*Oryctolagus cuniculus dom.*) has been widely employed as an animal model for studies of diet-induced hypercholesterolemia (183, 212–214), and its relationship to the pathogenesis of atherosclerosis. Such investigations have revealed strain differences in the response of this herbivore to cholesterol feeding (215, 216), and at the same time have indicated the rabbit's lipoprotein profile to be highly sensitive to dietary lipid. This characteristic is shared with the guinea pig, although the short term response of the two species to dietary cholesterol is quite different (90, 183).

Variable lipoprotein concentrations have been reported in rabbits fed normal, low fat (low cholesterol) diets, and these are summarized in **Table 18.** While VLDL levels are generally low and less than about 50 mg/dl, IDL is often elevated and present in amounts resembling those of LDL (of d 1.019–1.063 g/ml), i.e., of the order of 100 mg/dl. A wide range in IDL levels is apparent, and authors have failed to detect these substances (149). The combined concentrations of VLDL, IDL and LDL found by Camejo et al. (212) appear exceptionally low. Apart from the estimation of Kirkeby (152), HDL is uniformly the dominant lipoprotein class, levels typically being in the range of 180–250 mg/dl.

As compared to man and other mammals, the lipoprotein profile of the rabbit is relatively broad, ill-defined, and distinguished by significant amounts of IDL; these aspects are clearly evident from electrophoretic (92) and density gradient studies (216). The latter investigations reveal a distinct difference between the profile in fasted Red Burgundy and New Zealand rabbits. Animals of the Red Burgundy strain had levels of VLDL, IDL, and LDL (27, 37, and 65 mg/dl, respectively) that were twice as high as the New Zealand animals. On the other hand, HDL concentrations (186–191 mg/dl) were indistinguishable. Large amounts of lipids were detected in the fraction of d > 1.21 g/ml in both strains. Similar experiments, in which we used the gradient of Redgrave, Roberts, and West (218), were conducted in our own laboratory. The data suggest that the centrifugal conditions involved are insufficient for the complete recovery of HDL, which could therefore account for Pescador's findings (216).

The chemical compositions of the major serum lipoprotein classes in the rabbit are tabulated in **Table 19.** Although VLDL (d < 1.006 g/ml) was uniformly triglyceride-rich, the protein and cholesteryl ester contents (8.8–15% and 9.3–22.3%, respectively) varied considerably. Such variations did not appear to be at the expense of triacylglycerol, suggesting considerable differences in average particle size between the various preparations. Particle diameters ranged from 300–1000 Å in the fraction examined by Stange, Agostini, and Papenberg (219); their morphological appearance resembled that of human VLDL, thereby suggesting a similar overall molecular organization. Rabbit and human VLDL also appear alike in net surface charge, as manifested by the similar electrophoretic mobilities of corresponding fractions (212, 220); similar comments apply to the LDL and HDL of the two species.

TABLE 19. Chemical composition of rabbit serum lipoproteins (mean weight %)[a]

	VLDL					LDL				HDL			
Density limit (g/ml)	<1.006	<1.006	<1.006	<1.019	<1.019	1.006–1.063	1.006–1.063	1.006–1.063	1.019–1.063	1.006–1.070	1.063–1.21	1.063–1.21	1.070–1.21
Reference	(6)	(219)	(217)	(213)	(212)	(6)	(213)	(219)	(212)	(217)	(6)	(219)	(217)
Cholesteryl ester	22.3	11.5	9.3	5.1	4.3	35.7	25.1	43.4	24.4	29.7	20.8	10.7	15.5
Free cholesterol	4.5		10.7	9.1	8.9	5.4	11.1		9.1	9.8	2.5		2.3
Triglyceride	50.0	55.4	48	53.0	54.1	19.4	12.3	11.0	11.9	13.3	8.2	17.9	11.6
Phospholipid	14.5	21.6	17	17.3	17.2	15.8	25.8	17.2	26.5	18.8	16.5	26.4	19.4
Protein	8.8	11.5	15	18.5	19.5	24.9	29.5	28.4	28.1	28.4	51.9	45.0	51.2

[a] Fractions were isolated by sequential ultracentrifugation.

Rabbit fractions, including both VLDL and IDL (i.e., d < 1.019 g/ml), are intermediate in composition between VLDL itself and LDL. The low density lipoproteins are, however, characterized by their higher protein and cholesteryl ester contents; the contributions of triacylglycerol and cholesteryl ester to the overall particle are generally greater and lesser, respectively, than those of the corresponding components in human LDL (6, 65). Rabbit LDL appears slightly larger (mean 260 Å) (219) than that of man and some higher mammals (86, 145), although this finding of Stange et al. (219) could be explained by the contribution of IDL to the fraction of d 1.006–1.063 g/ml. Thus rabbit LDL particles, isolated in a narrow density interval (d 1.020–1.050 g/ml), display a similar diameter (mean 226 Å)[4] to that in man (d 1.024–1.045 g/ml; mean 217 Å (86)). Indeed, evidence for a similar overall size distribution of rabbit and human lipoproteins may be derived from their similar behavior and elution volumes on agarose gel columns (18). The composition of rabbit HDL is akin to that of man, although considerable variation in neutral lipid content is apparent (Table 19). These particles range from 90 to 150 Å in diameter and may be composed of several subunits (219): similar suggestions have been made for human HDL (4). The heterogeneity of rabbit HDL awaits evaluation.

The only data on the phospholipid composition of rabbit lipoproteins is from Rodriguez et al. (213), who indicate that while phosphatidylcholine is the major species in VLDL, the amount of sphingomyelin is low, and phosphatidylethanolamine accounts for more than 10% of the total. Such a distribution is distinct from that of human VLDL (65, 137). Palmitate, oleate, and linoleate are the principal fatty acids (20% or more) in all three classes of lipid esters in rabbit VLDL; stearate attained 23% in the phospholipids, however. Such fatty acid patterns are not unlike those typical of the human substances (65).

Despite the extensive experimental use of the rabbit, knowledge of its apolipoproteins is limited essentially to the protein moieties of VLDL and HDL, and is due mainly to Shore and Shore (183, 215). The electrophoretic pattern in urea-polyacrylamide gel of apo VLDL from control animals revealed at least eight components (215): one stained region (corresponding to a component of low mobility) was greatly increased in proportion to the rest in gels from the VLDL of cholesterol-fed animals (183). Using the large amounts of VLDL which occur in cholesterolemic rabbits, Shore, Shore, and Hart (183) isolated and characterized the apolipoprotein in question, which they denoted the arginine-rich protein; this is now known as apo E. The apolipoprotein occurred in two forms (fractions R2 and R3 from a DEAE-cellulose column), each of which contained carbohydrate and slightly more than 10 moles of arginine per 100 moles of amino acids (183, 215). In addition, a protein corresponding to human apo C-I in electrophoretic migration and amino acid composition was also isolated from the cholesterolemic VLDL of cholesterol-fed rabbits; this component is present in small amounts in control VLDL. A major component of rabbit apo VLDL appears to be a counterpart to human apo B, at least as judged by its elution in the void volume from Sephadex G-150 (183) and its failure to enter the separating gel on polyacrylamide gel electrophoresis (215). This supposition has recently been confirmed in our laboratory,[5] since the presence of an apo B-like component in rabbit VLDL and LDL has been demonstrated by the reaction with an antiserum to human apo B. Furthermore, the chemical and physical properties of this apolipoprotein, isolated by gel filtration chromatography in detergent-containing buffer, closely resemble those of the human B protein.

The apolipoproteins of rabbit HDL have been fractionated by Shore and Shore (215) and by Børresen (221) and Børresen and Kindt (222). The major protein component, purified either by ion-exchange

[4] Chapman, M. J. Unpublished observations.

[5] Goldstein, S., and M. J. Chapman. Unpublished observations.

(215) or gel filtration chromatography (222), is a counterpart to human apo A-I. This apolipoprotein is apparently polymorphic, existing in at least two forms which differ minimally in amino acid profile. Its molecular weight is 25,000 (222), and a carbohydrate moiety is lacking. Børresen and Kindt (222) consider that the rabbit apolipoprotein shows a greater similarity to canine than to human apo A-I. Comparison of the sequence of the first 31 amino acid residues at the NH_2-terminal end of rabbit apo A-I with that in the human protein revealed about 62% homology; a minimum of 13 base changes in the DNA sequences which encode these proteins was necessary to account for such dissimilarities (222). In the case of canine apo A-I, only ten base changes are required. The human apolipoprotein appears to differ from its analogue in the rabbit (222), dog (206), swine (183), chicken (138), and turkey (120), in the insertion of a proline residue in position 4 at the NH_2-terminal end. No analogue of human apo A-II has as yet been identified in rabbit HDL, although one of the minor components of its protein moiety may be a counterpart to the human C-III apolipoprotein (216).

While the (rabbit) arginine-rich protein appears to play a role in cholesterol transport and metabolism (183), via mechanisms as yet unknown, other minor apolipoproteins are responsible for the genetic polymorphism of HDL in this herbivore (223). The antigens involved, termed Hl 1 and R 67, appear to be transmitted as autosomal dominant traits, and to reside in two different polypeptides. Electrophoresis in SDS-polyacrylamide gel indicated that the Hl 1 antigen resided in a polypeptide of molecular weight 40,000, whereas the R 67 antigen was found in a smaller apolipoprotein, of molecular weight 17,000. These antigens have provided an approach to the study of the heterogeneity of HDL particles in the rabbit, and suggest that the R 67 antigen resides in molecules containing apo A-I, but that the Hl 1 antigen is present in a species from which both apo A-I and the R 67 polypeptide are lacking (223).

Proboscideans

The order Proboscidea has received little attention. A notable member of this group is the elephant, whose lipid and lipoprotein levels appear among the lowest of the mammals, being comparable to those of rodents and ungulates (91). Lipoproteins of β-mobility are apparently the major lipid carriers in elephant serum (91).

Cetaceans and Pinnipeds

Knowledge of the lipid transport systems in the former group, which includes the whales, dolphins, porpoises (order Cetacea), is rather scant, extending to the ultracentrifugal profile, concentrations, lipid composition, and particle size of the major lipoprotein classes. Similar information is available on the other major group of marine mammals, the Pinnipeds, which embodies the seals, sea lions and walruses. These data are due to Puppione, Nichols, and Forte and have been summarized by Calvert (140). It is notable that in one study of a Pinniped, the harbor seal (*Phoca vitulina*), Puppione et al. (168) could isolate lipoproteins in the interval d 1.04–1.06 g/ml (equivalent to S_f 0–3 and thus HDL_1) which displayed α-mobility, molecular weight (1.2×10^6) and particle size (140 Å) similar to the corresponding human substances (molecular weight and size 1.1×10^6 and 155 Å, respectively). The occurrence of such HDL_1 in marine mammals in general, and Pinnipeds in particular, remains to be assessed. While HDL appears to be the major serum lipoprotein class in all the aforementioned marine mammals, the relative proportion of LDL may vary considerably (140). Indeed, the elephant seal (*Mirounga angustirostris*) has recently been found to lack detectable amounts of LDL (224). By contrast, the predominant lipoproteins of Antarctic seal serum (*Leptonychotes wedelii*) appear to correspond to LDL, exhibiting β-mobility on cellulose acetate electrophoresis and accounting for 85% of the total lipoproteins (225).

The variability in the lipoprotein profile in seals, and particularly in individuals of the same species, was further evident in the studies of Dangerfield et al. (91). These authors found circulating lipid levels in phocids to be among the highest of the mammals, ranging from 1200 to some 3000 mg/100 ml, approached only by that found in bears. As in the harbor and elephant seals, (140, 224), α-lipoproteins predominated in the grey seal (*Halichoerus grypus*) (91), but β- and α-lipoprotein levels were alike in Baikal seals (*Pusa sibirica*) (91). Undoubtedly, the marine mammals constitute a group of great interest and further studies of their lipid metabolism are warranted. Thus, the possibility that their lipoproteins contain hydrocarbons and diacylglyceryl ethers, as do the Cyclostome, Elasmobranch and Chondrost substances, should be more fully evaluated, especially since certain cetacean tissues, such as the jaw, head, and blubber fat, contain large amounts of these compounds (226). Wax esters may also be present (226), and it would seem important to determine whether these are derived directly from the wax esters of the zooplankton (such as the calanoid Copepod *Calanus plumchrus* (227)) in their diet and transported as such, or whether they are synthesized in situ from fatty alcohols and fatty acids.

Ungulates

The ungulates are divided into two major orders, the Perissodactyla, or even-toed ungulates such as the horse (Equus sp.), donkey, tapir, and rhinoceros (e.g., Indian rhinoceros, *Rhinoceros unicornis*), and the Artiodactyla, the larger of the two and divided into three sub-orders. These sub-orders are the Suiformes, as exemplified by the pig (*Sus domesticus*), the Tylopoda which includes the camels (e.g., the Bactrian camel, *Camelus bactrianus*), and the Ruminantia, of which the major representatives are the cattle (Bos sp.), sheep (*Ovis aries*), goats (e.g., *Capra hircus*), and deer (e.g., the wapiti, *Cervus elaphus*).

Perissodactyla. Relatively few observations have been made on members of this order, and information up to 1975 has been discussed by Calvert (140). Of the nine species examined by Leat et al. (148), HDL predominated in representatives of the family Equidae, i.e., the wild horse (*Equus przewalski*), the mountain zebra (*Equus zebra*), the common zebra (*Equus burchelli*), domestic horse (*Equus caballus*), donkey (*Equus asimus*) and onager (*Asinus hemionus*), in which it amounted to about 70–90% of the total lipoproteins exclusive of VLDL. By contrast, high density lipoproteins could not be detected, either by ultracentrifugal or electrophoretic methods, in members of the family Rhinocerotidae, i.e., the white rhinoceros (*Ceratotherium simum*), the black rhinoceros (*Diceros bicornis*), and Indian rhinoceros (*Rhinoceros unicornis*). These herbivores appear to transport the major portion of their lipids in an apparently complex lipoprotein of low density and β-mobility (148); presumably however, the presence of trace amounts (<10 mg/dl) of HDL, as found for example in the guinea pig, cannot be excluded. In the Tapiridae, represented by the Malayan tapir (*Tapirus indicus*), high density (α-) and low density (β-) lipoproteins were present at similar levels. It is thus apparent that there is no uniformity in plasma lipoprotein distribution among the Perissodactyla, even given their similar (non-ruminant) digestive physiology.

Artiodactyla. Among the even-toed ungulates (order Artiodactyla), the camel (*Camelus bactrianus*) appears unique in having a lipoprotein profile in which only minor amounts of HDL (e.g., 11 mg/dl (6)) are identifiable, the major compounds being LDL which attains concentrations slightly in excess of 100 mg/dl (6, 228). The density of such LDL was clearly less than 1.063 g/ml, (and not greater as is essentially the case in the guinea pig (6, 192)). VLDL is present at low levels (6); this did not, however, prevent Mills and Taylaur (6) from analyzing this lipoprotein. Being concentrated at the S_f 20 boundary, it was not surprising that camel VLDL showed the highest protein content of the 18 species examined by the latter investigators. Indeed, the proportion of protein in VLDL and LDL was indistinguishable (ca. 20%); although VLDL was triglyceride-rich (56%), LDL was enriched in cholesteryl ester (37%). The compositions of camel LDL and HDL were similar, (with the exception of a slightly higher protein content in 'HDL'), an observation consistent with the suggestion that this herbivore lacks a true class of high density substances (6, 228) and that its LDL and 'HDL' form a continuum of particles, enveloping the 1.063 g/ml "boundary." Little additional information (140) on the serum lipoproteins of sheep, goats, or deer has appeared in recent years. Thus, Leat and Northrop (228) confirmed that HDL constitutes the principle class, (accounting for 80% of the total), in sheep, goats, and deer (the wapiti and moose, *Alces alces*). These findings corroborate the small VLDL peak seen by Fried et al. (116) on analytical ultracentrifugal analysis of deer serum, and the presence of HDL in amounts three-fold greater than those of LDL (207 and 66 mg/100 ml respectively).

Significant progress has however been made in our knowledge of the predominant lipid-carrying molecules in cattle. Jonas (87, 229–233) and Stead and Welch (234–236) have described exhaustive physicochemical studies of the HDL of the cow (Bos sp.). In ultracentrifugal studies of bovine serum, Jonas (229) found the flotation behavior of the HDL to be quite distinct from that of man. These macromolecules, which constituted about 80% (by weight) of the total lipoproteins of $d < 1.21$ g/ml, were present within the density interval 1.063–1.125 g/ml. They were thus equivalent to the fraction denoted as HDL_2 in man, although the bovine lipoprotein floated faster (229). The remaining material was present as substances of $d < 1.063$ g/ml, with only trace amounts of HDL_3. Bovine HDL appeared rather homogeneous by several criteria, including its resolution as a single band on electrophoresis and its behavior as a single peak in sedimentation velocity measurements. Bovine HDL attained concentrations averaging 440 mg/dl; similar levels have been noted by other investigators, e.g., 341 mg/dl (6) (separated ultracentrifugally and quantitated chemically, and lacking correction for losses during isolation) and 400–650 mg/dl (237) (calculated assuming an HDL protein content of 50% by weight). HDL also appears to be the major class in other cattle, such as the bison (*Bison bonasus*), domestic ox (*Bos taurus*) and musk ox (*Ovibos moschatus*) (228). The average molecular weight of bovine HDL, estimated by sedimentation equilibrium studies, was 376,000 (229), closely resembling that of human

HDL_2 (4, 137). Employing both approach-to-equilibrium and equilibrium methodologies, Stead and Welch (235) found a rather higher value for a homogenous HDL (d 1.060–1.21 g/ml) fraction from Friesian cows, i.e., 567–576 × 10^3. The latter workers suggested that such differences arose from the higher value of partial specific volume (1.1 g/ml) used by Jonas (229); the value which they determined (1.071 g/ml) was similar to that seen in a bull (1.078 g/ml) (238) and in Hereford steer calves (1.077 g/ml) (239). The partial specific volume of bovine HDL (0.934 ml/g) (235) was higher than that of both the HDL_2 and HDL_3 of man ((136), 0.905 and 0.867 ml/g, respectively).

The chemical composition of bovine HDL is highly variable (**Table 20**), and particularly in its proportions of protein (range 28.4–43.5%), phospholipid (18.6–40.8%), and triglyceride (0.1–19.9%); only the cholesteryl ester content was relatively constant (25.4–31.1%). Bovine HDL lipoprotein shared little in common with either subfraction of human HDL (Table 20), an observation noted previously by both Jonas (229) and Stead and Welch (234). Indeed, bovine HDL could be readily distinguished by the elevated proportion of cholesteryl ester. The lipoproteins of density less than 1.063 g/ml in bovine serum are rather complex in nature and distribution, as remarked by Calvert (140), and cannot be resolved simply by ultracentrifugal means. Use of precipitation, ultracentrifugal, and electrophoretic procedures allowed Stead and Welch (234) to separate VLDL (d < 1.019 g/ml), LDL_1 (d 1.019–1.039 g/ml), and LDL_2 (d 1.039–1.06 g/ml) fractions whose chemical compositions are in Table 20. VLDL, (including IDL, and only amounting to a minor fraction), was triglyceride-rich as in other mammals, but in addition, displayed an elevated phospholipid content. On the other hand, VLDL was deficient in cholesteryl ester and comparable to rat VLDL in this respect (Table 15). The bovine LDL_1 subfraction was especially rich in phospholipid (41.2%) and thus quite unlike the human fraction of similar density range (LDL-IV; d 1.030–1.040 g/ml (43)); nonetheless, the human and bovine LDLs were of similar protein and cholesteryl ester content. The LDL_2 characterized by Stead and Welch (234) could only be distinguished from LDL_1 by the lower proportion of phospholipid in the former. Little resemblance is apparent between the bovine fraction of d 1.006–1.063 g/ml (6) and either LDL_1 or LDL_2 (234); in particular its protein content was elevated, equalling that of the HDL fraction of d 1.060–1.21 g/ml (234). Presumably differences in nutritional, gestational, and lactational status between donor cows, which are known to affect lipoprotein profile (237), may account for these discrepancies.

It is not apparent whether the fraction denoted 'LDL_2' by Stead and Welch (234) was truly an apo B-containing lipoprotein, or whether it corresponded to the HDL_1 species isolated in the density range 1.04–1.06 g/ml by Puppione et al. (168); both groups used serum from lactating cows. Thus, both studies lacked data on the apolipoprotein content of the respective fractions and further comment must await additional investigations along these lines. Comparison of the chemical composition of rat HDL_1 and bovine LDL_2 (Tables 15 and 20) reveals a marked degree of resemblance however, suggesting some structural equivalence between them. The fatty acid patterns of the lipid esters of bovine VLDL, LDL_1, LDL_2, and HDL (234) are, as might be expected, somewhat of a reflection of the digestive physiology of this ruminant. Thus, with the exception of the cholesteryl esters of LDL_1, LDL_2, and HDL, the bovine lipids were substantially more saturated than their human counterparts (65), presumably reflecting hydrogenation occurring in the rumen. The cholesteryl esters of the LDL_1, LDL_2, and HDL fractions contained elevated levels of the 18:2 and 18:3 acids, with linoleate accounting for 55–60% of the total. Such a high 18:2 content in these cholesteryl esters is apparently due to the substrate specificity of bovine LCAT activity (240). In addition, phytanic acid was

TABLE 20. Chemical composition of bovine serum lipoproteins (mean weight %)[a]

	VLDL	LDL			HDL		
Density limit (g/ml)	<1.019	1.019–1.039	1.039–1.060	1.006–1.063	1.063–1.21	1.060–1.21	1.063–1.125
Reference	(234)	(234)	(234)	(6)	(6)	(234)	(239)
Cholesteryl ester	3.7[b]	32.3[bc]	39.3[bc]	41.2	31.1	29.5[b]	25.4
Free cholesterol	4.9	4.9	5.7	11.2	3.7	1.2	4.1
Triglyceride	60.2	0.1	1.8	6.8	4.4	0.1	19.9
Phospholipid	25.1	41.2	29.2	21.5	21.3	40.8	18.6
Protein	6.0	21.3	24.0	19.4	43.5	28.4	32

[a] Fractions were isolated by sequential ultracentrifugation unless otherwise stated.
[b] Calculated from ref. 234.
[c] Isolated by gradient electrophoresis from LDL of d 1.019–1.060 g/ml.

present in the triglycerides and phospholipids of LDL_1, LDL_2, and HDL.

The early studies of Jonas (229) indicated that the apoprotein of bovine HDL was less complex than that of its human counterpart (4), thereby resembling apo HDL in the dog (203). Subsequently, an apolipoprotein analogous to human apo A-I in molecular weight (27,000) and amino acid composition was found to constitute about 90% of the total protein moiety; like the human protein, bovine apo A-I displayed microheterogeneity. In addition to this apolipoprotein, bovine apo HDL contains about 12% (by weight) of other components, with molecular weights of 13,000 and 11,000 on electrophoresis in SDS-polyacrylamide gel. They were distinct in amino acid composition from bovine apo A-I, and it could not be decided whether one of them might bear a relationship to human apo A-II. Bovine apo A-I appears to resemble its human counterpart in possessing aspartic acid as its N-terminal residue (236).

Like human apo A-I, the bovine polypeptide possessed a remarkable ability to form relatively stable aggregates (232), whose structure and composition varied little over the concentration range 5×10^{-7} to 5×10^{-4} M. The intermolecular forces involved appeared rather specific and electrostatic interactions were eliminated, but could be overcome by the introduction of molecules with amphipathic properties, (e.g., SDS), or by agents which disrupt hydrogen bond formation (e.g., urea).

Difference absorption spectroscopic studies of native bovine HDL (231) indicate a large proportion of the tyrosine (75%) and tryptophan (40%) residues to be exposed to the solvent, with the resultant values higher than those considered typical of globular proteins. These results have been interpreted by Jonas (231) as indicating that most of the protein component of bovine HDL is located at its surface, and implying, at the same time, some degree of specificity in lipid–protein interactions. This hypothesis is entirely consistent with the amphipathic helix proposed for (human) apo A-I by Segrest et al. (241). Finally, recent recombination studies of bovine apo A-I with phospholipid (1,2 dimyristoyl-*sn*-glycero-3-phosphocholine) and cholesterol suggest a preferential association of apolipoprotein and phospholipid, to the extent that the lipid layer immediately adjacent to the polypeptide is constituted exclusively of phospholipid (242, 243). While it is evident that some of the structural features of bovine lipoproteins, and particularly HDL, are established, further work is clearly needed to determine the apolipoprotein content of lipoproteins of density less than 1.063 g/ml, and to assess the relationship between lipoprotein profile and the sex of the animals, their age (immature or adult) as well as the period of gestation/lactation in cows. Furthermore, the role of the various lipoproteins (and chylomicrons) in the production of milk by these ruminants should be evaluated.

The pig (*Sus domesticus*) has been judged a suitable experimental animal for atherosclerosis research on the basis of several criteria, among which is the resemblance of its serum lipoproteins to those of man. Calvert (140) reviewed the relationship between the two species primarily with respect to the LDL class. Subsequent to his discussion, it has become evident that, in addition to the marked heterogeneity of porcine LDL and the lack of an apo A-II-like component in its HDL, there are several aspects of the physical and chemical properties of porcine serum lipoproteins which serve to distinguish them from their human counterparts. Moreover such dissimilarities have been consistently documented in the reports of several workers in this field.

One essential distinction between the porcine and human lipoprotein systems, which has to some extent been neglected, is the density distribution of the porcine low density (apo B-containing) and high density (apo A-containing) lipoproteins. Thus, Janado, Martin, and Cook (244) first demonstrated that porcine LDL was distributed up to a (solvent) density of 1.090 g/ml. This finding has subsequently been confirmed by Mahley and Weisgraber (143), who found LDL distributed principally in the interval 1.03–1.08 g/ml; HDL extended to a lower limiting density of 1.070 g/ml. In detailed studies of seven LDL subfractions isolated within the overall range 1.006–1.090 g/ml, Fidge and Smith (245) confirmed these observations and extended them to reveal a skewed particle distribution due to a major contribution from LDL of d 1.063–1.074 g/ml. Apo A-containing lipoproteins were detected in the subfractions of d 1.006–1.019 and 1.074–1.090 g/ml, leading these investigators to the suggestion that pig LDL should be separated within the range 1.019–1.074 g/ml. Approximately 95% of the apoprotein of LDL of d 1.030–1.074 g/ml consisted of an apo B-like component, suggesting the existence of a relatively homogeneous lipoprotein species (Lp B, for example) in this density range. These findings agree with those of Jackson et al. (246) who showed that the apoproteins of LDL_1 (d 1.020–1.060 g/ml) and LDL_2 (d 1.060–1.090 g/ml) were essentially identical, and with those of Knipping, Kostner, and Holasek (247), who could isolate a lipoprotein (Lipoprotein B) containing only apo B from the density range 1.015–1.080 g/ml by gel filtration chromatography.

The major apolipoprotein of pig LDL (d 1.024–

1.045 g/ml) isolated by gel filtration chromatography in detergent-containing buffer was analogous to human apo B (86). The human and pig proteins behaved similarly upon electrophoresis in SDS-polyacrylamide gel (molecular weight > 250,000) (86) and were markedly alike in amino acid composition (86, 247); in addition, the pig apolipoprotein cross-reacted immunologically with antiserum to human apo B (80). This apolipoprotein is also a major component of porcine apo VLDL (247), accounting for about 50% of protein mass. There is a good deal of similarity in the chemical compositions described for the various density fractions of porcine LDL (86, 143, 245, 246); rather less data are available on VLDL (143, 248, 249), which is typically a minor or even trace component of porcine plasma (6, 116, 244, 250). Immunological studies first indicated that human and pig LDL might differ in their carbohydrate moieties (86), as indeed do VLDL from these two species (251). Recently, Azuma and Komano (251) have documented dissimilarities between the major glycopeptides of porcine LDL (d 1.007–1.090 g/ml) and those of man. Three types of glycopeptide were isolated from a pronase digest of porcine apo LDL and they were partially characterized. Although the composition of the neutral glycopeptide resembled one of the carbohydrate units seen in human apo LDL (253), the second type, acidic in nature, was slightly different (251, 253). Analytical data suggested that pig apo LDL possesses 11–28 carbohydrate units per mole (252).

In addition to apo B, the low density (d < 1.063 g/ml) lipoproteins of pig serum possess a number of low molecular weight apolipoproteins (247, 248, 250, 254, 255). However it appears that these polypeptides, which elute on gel filtration chromatography in a similar position to the human C apolipoproteins, are distinct in distribution and properties to the human peptides. The major apolipoprotein seems to be analogous to human apo C-II as judged by its identical migration on polyacrylamide gel, similar amino acid composition and molecular weight, and ability to activate a purified lipoprotein lipase preparation (4, 247, 254); the carboxyl-terminal amino acid of this protein is serine (247, 254). For unaccountable reasons, the same peptide isolated by Fidge (255) had only low cofactor activity as compared to human apo C-II. The additional, minor peptides of porcine apo VLDL represent analogues of the human C-I, C-III-1, C-III-2, and arginine-rich (apo E) apolipoproteins (247, 250, 255). However the pig fractions, which migrate electrophoretically in a similar position to the human C-III peptides, differ little from porcine apo C-II in amino acid composition (250); they also lack sialic acid (250, 255). Their homology with human apo C-III is therefore tentative, and the nature of these peptides remains to be clarified. No immunological cross-reactivity could be detected between antisera to pig apo C-II and human apo C-II (246), nor between human and pig apo C-I (250). The presence of an analogue to human apo E has however been reported by Knipping et al. (250), who isolated this polypeptide from porcine VLDL, and by Mahley et al. (184) who used the large amounts of β-migrating VLDL and HDL_c in cholesterol-fed swine as rich sources of starting material for the purification of this apolipoprotein. The porcine protein resembles that of man in amino acid composition and coelectrophoreses with it (181, 184, 250); nonetheless, it did not react with antiserum to human apo E (250).

HDL in the pig is distinct from that in man in being rather homogeneous and displaying essentially a single component (244, 250, 256, 257), with a molecular weight of ca. 210,000. It is the predominant lipoprotein of porcine plasma, present at levels ranging from 100–180 mg/dl (6, 116, 250) and representing 50–60% of the total lipoproteins of d < 1.21 g/ml. Relatively few analyses of its chemical composition have been reported, and these tend to be rather variable (**Table 21**). As noted previously, (140), an apolipoprotein analogous to human apo A-I predominates in the protein moiety of pig HDL (184, 247, 250, 258), although the porcine protein is

TABLE 21. Chemical composition of porcine HDL (mean weight %)[a]

	Density Limit (g/ml)				
	1.063–1.21	1.09–1.21	1.063–1.12[b]	1.063–1.12	1.12–1.21
Reference	(6)	(249)	(248)	(248)	(248)
Cholesteryl ester	26.0	14.1	22.9	27.4	19.0
Free cholesterol	3.9	2.6	3.4	4.1	2.2
Triglyceride	2.0	n.d.[c]	2.0	3.8	2.9
Phospholipid	22.4	27.4	38.3	37.3	33.3
Protein	45.8	54.8	33.4	27.4	42.6

[a] Fractions were isolated by ultracentrifugation unless otherwise stated.
[b] Isolated by dextran sulfate precipitation followed by ultracentrifugation (248).
[c] n.d., not determined, estimated at 1% (249).

distinct in C-terminal sequence (247, 258). Like its human counterpart, pig apo A-I could activate the lecithin:cholesterol acyl transferase of human serum (258); moreover, pig and human apo A-I cross-reacted immunologically (250). Pig apo HDL also contains some minor polypeptides, including apo C-II (250).

Recombination studies of the apoprotein of porcine HDL with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine have been described (259, 260). After sonication of apoprotein and phospholipid, the recombinant structures were purified by gel filtration chromatography and studied by several physicochemical techniques, including small angle X-ray scattering, differential scanning calorimetry, nuclear magnetic resonance (NMR) spectroscopy, electron spin resonance spectroscopy, and circular dichroism. The results obtained suggest that, as in native HDL, the surface of the lecithin:apoprotein complex consists of a mosaic of lecithin polar groups and protein (261). In much the same manner as Jonas and colleagues (242, 243) concluded in the case of recombinants of bovine apo A-I, the structure of the complex appeared directed by the fundamental bilayer organization of the lecithin. Furthermore, it was suggested that the apoprotein was organized into an amphipathic, α-helical conformation (see review (4)). The same group of investigators also employed NMR spectroscopy to investigate the molecular organization of porcine LDL and HDL (262). In the case of LDL, these studies led to the proposal of a trilayer model in which a portion of the apoprotein was organized in a core at the center of the particle; this structure resembled the early model proposed for human LDL in 1972 by Mateu et al. (263). Subsequent studies employing ^{13}C NMR (264) indicated that not only the cholesterol ring system in LDL and HDL, but also the fatty acyl chains in these lipoproteins are restricted in their rotational mobility to a greater extent than originally envisaged (262).

An important contribution to our knowledge of the structural organization of the hydrophobic, cholesteryl ester-containing core of serum lipoproteins has been made from studies of HDL_c, a cholesteryl ester-rich lipoprotein which may be isolated from the serum of pigs fed a high-cholesterol, saturated-fat diet (184). This lipoprotein has α_2-mobility, contains apo E and apo A-I in its protein moiety, and is intermediate in size between LDL and HDL (184). Evidence from differential scanning calorimetric and X-ray small angle scattering investigations shows that the cholesteryl esters of HDL_c may form a microdomain with a detectable, reversible thermal transition (265), similar to that first seen in LDL (266). Since HDL lacks this order–disorder transition, (itself attributable to a change in the organization of cholesteryl esters from a smectic-like to a more disordered structure (266)), it appears that HDL_c may represent the lowest size limit at which the cholesteryl esters in lipoprotein particles may assume an organized structure (265). Indeed, HDL_c (diameter ~ 180 Å) seems to possess one less molecular unit of cholesteryl ester than LDL (diameter ~ 220 Å) in its radially-repeating core organization; thus, the outer steroid layer in HDL_c overlaps at a radius of 45 Å, whereas this occurs at 60 Å in LDL, the difference corresponding approximately to the length of a single cholesteryl ester molecule. Furthermore, analysis of the electron density maxima, together with model calculations, suggested some common structural arrangement and interaction as regards the surface components (protein and phospholipid) and outer layer of cholesteryl esters (265) in both LDL and HDL_c. This has led to the suggestion that such an interaction may be responsible for the stabilization of the surface structure of cholesteryl ester-transporting lipoproteins (265).

Non-human Primates

Apart from the rat and pig, the non-human primates have proven the most popular animals for the study of the influence of diet on serum lipid and lipoprotein profile and of the relationship between these factors and the atherogenic process. Despite this, data in the literature extend in only a few cases to the characterization of the serum lipoproteins in animals fed a normal diet. Calvert (140) has summarized some of the problems encountered in primate studies, among which the small number of animals often employed is of special concern. His review also contains extensive information on the varying susceptibility of different species to dietary lipid and sterols (principally cholesterol).

Old World Monkeys. The Old World monkeys, (family Cercopithecoidea) are phylogenetically closer to man than those of the New World group (family Cebidae). However for reasons which will become evident, it may become increasingly desirable to use certain species of New World monkey instead of Old World varieties, given the fact that the former are more easily handled, breed readily in captivity, and may be less expensive to maintain. Of the Old World monkeys, Macaque species and particularly the rhesus macaque (*Macacca mulatta* or *M. rhesus*), have received by far the most attention. Indeed, Scanu and coworkers (267–270) have described extensive investigations of the structure of the low and high density lipoproteins in this species, in which HDL

accounts for ca. 70% of the total lipoproteins of d < 1.21 g/ml (269). The findings of Fless and Scanu (267), Chapman and Goldstein (86), and Lee and Morris (271) are consistent in indicating a close resemblance between the LDL of the rhesus monkey and man. There appear however to be some minor differences between the two in size, (the rhesus LDL being of slightly smaller diameter (86,267)), and in degree of hydration (267). Variable estimates of the molecular weight of rhesus LDL have been reported, ranging from 2.3×10^6 (267) to 3.3×10^6 (271); the donor animals received chow diets and LDL was isolated within differing density intervals (1.019–1.050 g/ml, from which a fraction of d 1.032–1.036 g/ml was removed (267), and 1.019–1.063 g/ml (271)). Such a discrepancy could be explained by a skewed distribution of LDL particles towards the 1.019 g/ml limit in the latter study; some variability has also been detected between individual animals (268).

Recently, further information on the heterogeneity of rhesus LDL has become available (272). By application of isopycnic and rate zonal density gradient centifugation, three subfractions (LDL-I, LDL-II, and LDL-III) could be isolated from half of the normolipidemic animals studied; some of the physical properties of these particles differed, and these included hydrated density (1.027, 1.036, and 1.050 g/ml, respectively), molecular weight (3.32, 2.75, and 3.47×10^6, respectively) and particle size (243, 212, and 240 Å, respectively). The chemical compositions reported for rhesus LDL and its subfractions (**Table 22**) display an overall similarity, the largest variation occurring in triglyceride. Moreover, monkey and human LDL are alike. It is perhaps pertinent to note at this juncture that ultracentrifugal studies showed that the major component of rhesus LDL could be recovered in the density interval 1.024–1.045 g/ml (86), a finding consistent with the density gradient data (267). The relative proportions of the major particle species appear however to vary from individual to individual (272); these species differ primarily in protein content (range 19.6–26.6%). The similarity between human and rhesus LDL includes the composition of their phospholipids (65, 137, 268, 271, 272) in which phosphatidylcholine and sphingomyelin are the major components. In one study (268), large amounts (11.1%) of phosphatidylethanolamine were found. The fatty acids of the lipid esters of LDL displayed a similar pattern to those of man (65, 268, 271) although minor dissimilarities in phospholipid fatty acids were apparent.

While several authors have analyzed the amino acid composition of the whole apoprotein (i.e., apo LDL) of rhesus LDL (267, 271) (thereby including both its major apoprotein and small amounts of low molecular weight apolipoproteins (86)), only one group (86) appears to have isolated the major apolipoprotein by gel filtration chromatography and examined its physical, chemical, and immunological properties. This protein is a counterpart to human apolipoprotein B on the basis of its amino acid composition and molecular weight in SDS-polyacrylamide gel, but could be distinguished from the human protein by immunological techniques. Thus, rhesus apo B cross-reacted in a micro-immunoprecipitation test with an antiserum to human apo B to the extent of ca. 84% of the reaction between the human apolipoprotein and its homologous antiserum. This is consistent with the partial identity seen between human and rhesus LDL on immunodiffusion (86, 127, 267). It is also noteworthy that rhesus apo B represents about 95% of the protein moiety of the native LDL, and like its human analogue, is a glycoprotein (267). Interestingly, one of the major differences in the LDL subspecies characterized by Fless and Scanu (272) was the increasing carbohydrate content of their protein moiety as the parent particle gained density. Whether this is a primary factor in the varying conformation of the apoprotein in the surface of these particles (LDL-I–LDL-III) is as yet unknown. The overall

TABLE 22. Chemical composition of rhesus monkey and human LDL (mean weight %)[a]

	Monkey (*M. mulatta*)							Man
Density limit (g/ml)	1.019–1.050	1.019–1.050	1.024–1.045	1.019–1.063	LDL-I 1.027[b]	LDL-II 1.036[b]	LDL-III 1.050[b]	1.024–1.045
Reference	(267)	(268)	(86)	(271)	(272)	(272)	(272)	(86)
Cholesteryl ester	36.0	35.7	33.3	32.8	37.5	37.3	34.6	38.9
Free cholesterol	14.0	11.0	12.2	7.8	8.1	7.8	7.4	8.8
Triglyceride	2.5	4.8	6.3	9.4	8.6	7.6	7.1	6.3
Phospholipid	23.6	25.2	23.1	23.1	26.1	25.3	25.3	22.8
Protein	24.0	23.2	25.1	22.9	18.5	20.8	24.0	23.2

[a] Fractions were isolated by sequential ultracentrifugation except as noted.
[b] Hydrated density was determined by density gradient ultracentrifugation. Protein-bound carbohydrate represented 1.1, 1.2, and 2.6% (by weight) of LDL-I, LDL-II, and LDL-III, respectively.

molecular organization of these subspecies appears indistinguishable (272), i.e., they conform to the model proposed by Shen, Scanu, and Kezdy (52). The HDL_2 and HDL_3 subclasses of rhesus LDL, isolated by ultracentrifugal flotation in the density intervals 1.063–1.125 and 1.125–1.21 g/ml, respectively, appeared homogeneous by chromatography on agarose gel and by analytical ultracentrifugation (269). The molecular weights were 3.9×10^5 for HDL_2 and 1.97×10^5 for HDL_3, values reminiscent of their human counterparts (4, 137); a similar resemblance was seen in the particle diameters of HDL_2 (118 Å) and HDL_3 (75 Å) in the two species (95 and 75 Å, respectively, in man, (273)). The hydrodynamic properties of corresponding subfractions in rhesus and man also appeared comparable (269).

The chemical compositions of the rhesus HDL_2 and HDL_3 markedly resembled those of the corresponding human lipoproteins. Phosphatidylcholine was the major phospholipid species in all fractions, although the sphingomyelin content of the monkey substances (3.8%) was substantially lower than that of the human fractions (14.5% in HDL_2 and 9.2% in HDL_3) (137, 269). The monkey and human high density subfractions are thus alike in chemical and physical properties; they also cross-react immunologically. The rhesus monkey was, however, distinct in exhibiting elevated quantities of HDL_2; these represented 45% of the total lipoproteins ($d < 1.21$ g/ml), giving an HDL_2:HDL_3 ratio of 2:1 (269) instead of 1:3 as typically seen in man (65).

As in man, two polypeptides predominated in rhesus HDL_2 and HDL_3. These were found upon purification and characterization to represent homologues to the human A-I and A-II apolipoproteins (270, 274). Some rather important differences are however to be noted. Fraction III, or rhesus apo A-I (270), was a single polypeptide chain of 27,000 molecular weight, with NH_2-terminal aspartic acid and -COOH terminal, glutamine. While existing in (two) polymorphic forms, rhesus apo A-I could be distinguished from the human protein in its amino acid composition (higher content of glutamate and lower proportion of arginine). The rhesus apolipoprotein did however cross-react immunochemically with human apo A-I. Recently rhesus apo A-I has been found in fluorescence polarization studies to act like a macromolecular surfactant in its ability to form small, soluble complexes with sonicated dispersions of egg phosphatidylcholine (275). The structural properties which endow rhesus apo A-I with this capacity await evaluation.

The second major polypeptide of rhesus apo HDL, fraction IV (i.e., apo A-II), consisted of a single polypeptide chain of molecular weight 8500 with a blocked NH_2-terminus, (pyrrolidone carboxylic acid) and COOH-terminal glutamine (270). Antiserum to this apolipoprotein gave a single precipitation line with human apo A-II. Studies of the covalent structure of rhesus apo A-II subsequently established its sequence (77 residues) and revealed the substitution of a serine residue at position 6 for cysteine in human apo A-II (274). While a high degree of homology was evident in the two proteins, an additional five conservative replacements had occurred, (at residues 3, 40, 53, 59, and 71), in the rhesus polypeptide. The substitution of serine at position 6 in rhesus A-II clearly accounts for its monomeric structure (274).

Recently, Rudel, Greene, and Shah (276) have described the separation and characterization of the plasma lipoproteins in rhesus monkeys fed a low cholesterol (0.05 mg/Kcal)-high fat (25% w/w lard) diet. In its distribution of calories, this regimen approximated that of the typical North American diet. The lipoprotein profile in these animals was complex, and six fractions could be distinguished by their size, density, and electrophoretic mobility. Evidence was obtained for the presence of β-migrating VLDL of the type described by Mahley and colleagues in cholesterol-fed swine (184), rats (13), dogs (182, 207), and monkeys (185). The question as to whether one may truly address these animals as "control diet-fed" is a matter of debate; these studies do perhaps suggest that our knowledge of the rhesus lipoprotein spectrum under conditions of a low-fat diet may be incomplete.

Some data on the LDL of cynomolgus monkeys (*Macaca fascicularis*) have also been reported (277). The chemical composition of this LDL was: 22.8% protein, 23.3% phospholipid, 2.0% triglyceride, 8.5% free cholesterol, and 43.4% cholesteryl ester, thereby resembling that of rhesus and human LDL (Table 22). The molecular weight of this particle (3.29×10^6) was in the range seen for human LDL (4); it displayed a reversible thermal transition as previously documented in human LDL (266) and porcine HDL_c (265). Moreover, the cholesteryl ester transition temperature was correlated with the degree of saturation of LDL cholesteryl esters. There was no correlation between the enthalpy of this transition and particle molecular weight within the range 3 to 7 $\times 10^6$. These results were interpreted as indicating that LDL cholesteryl esters in the (control) monkeys were structurally organized, (at body temperature, and thus above the transition point), into a radially-orientated, nematic-like arrangement, as occurs in human LDL (277).

Some aspects of the serum lipoprotein profile in

baboons (Genus Papio) have been reviewed, and compared to those in the rhesus monkey and chimpanzee, by Blaton and Peeters (278). As in the rhesus, HDL is the major lipoprotein class. Scant information is at hand to date on the chemical composition of baboon serum lipoproteins, on the possible heterogeneity of particle species, and on their respective density distributions. Nevertheless, the composition of the major lipoprotein classes isolated in the conventional density intervals suggests a close relationship between the baboon and human HDL (**Table 23**). Baboon VLDL, present only in small amounts, is rich in cholesteryl ester but poor in triglyceride (279); such substitutions of neutral lipid in VLDL are typically a function of diet. On the other hand, the LDL of this primate was rather richer in triglyceride and poorer in cholesteryl ester than human LDL (see Tables 21 and 23). This may give rise to the slightly larger size of baboon LDL (mean diameter 245 Å (86)). The major apolipoprotein of this particle is analogous to human apo B in amino acid composition and apparent molecular weight (86); baboon LDL (and apo B) cross-reacts with antisera to human LDL and to human apo B (80, 86, 173, 279), giving reactions of partial identity.

Baboon HDL, of density 1.063–1.21 g/ml, bears an overall resemblance to that of man in chemical composition (Table 23), (with the exception of an elevated triglyceride content seen in one study (279). Like rhesus HDL, HDL_2 levels predominated over those of HDL_3 in the ratio 1.6:1 (278). The phospholipids of the baboon HDL were poor in sphingomyelin and enriched in phosphatidylethanolamine (278, 280); no structural significance has as yet been attached to this observation. Only minor differences were seen in the fatty acid profiles of baboon and human HDL (280). The major apolipoproteins of baboon HDL are homologous with human apo A-I and apo A-II (278, 280). Two substitutions have been detected in the first 30 amino acid residues at the NH_2-terminus of baboon A-I; its COOH-terminal was glutamine. As in rhesus HDL, the baboon lipoprotein contained a monomeric form of apo A-II with a molecular weight of 8500; this polypeptide lacked cysteine.

Microcalorimetric evaluation of the recombination of the baboon HDL apolipoproteins with purified phospholipids revealed that the thermodynamic properties of binding differed minimally from those of human HDL (280). Immunological studies have provided evidence for the presence of additional apolipoproteins, analogous to the human C-I, C-II, and C-III peptides (279) in baboon lipoproteins. Such apolipoproteins probably correspond, at least in part, to the peptides detected upon electrophoresis of the tetramethylurea-soluble fraction of baboon apo LDL (86).

Apart from the rhesus macaque, the only other Old World primate in which the distribution of lipoproteins and apoproteins has been exhaustively studied is the Patas monkey, (*Erythrocebus patas*) (147). Employing an initial ultracentrifugal separation followed by Pevikon block electrophoresis, Mahley et al. (147) isolated four fractions which floated in the following ranges: VLDL, d < 1.006 g/ml; LDL-I, d 1.02–1.06 g/ml; LDL-II, d 1.05–1.085 g/ml; and HDL, d 1.07–1.21 g/ml. VLDL was triglyceride-rich, but contained more phospholipid and less cholesterol than human VLDL: such particles represented only 1% of the total, and ranged in size from 300 to 850 Å. The principle apolipoprotein was an apo B-like component; analogues to human apo E and the apo Cs were also detectable.

The LDL-I and LDL-II fractions, resolved by preparative electrophoresis, overlapped in density distribution and were similar in chemical composition, both possessing high contents of cholesterol (ca. 45%). Levels of LDL-I (ca. 120 mg/dl) were about four-fold higher than those of LDL-II. As might be anticipated, these lipoproteins were of similar size, being in the range of 180–300 Å. Their major protein component was the B apoprotein, but LDL-II also contained small amounts of proteins with the mobility of apo E and apo A-I upon electrophoresis (on the basis of both charge and size). These observations

TABLE 23. Chemical composition of baboon serum lipoproteins (mean weight %)[a]

	VLDL	LDL		HDL	
Density limit (g/ml)	<1.006	1.006–1.063	1.024–1.045	1.063–1.21	1.063–1.21
Reference	(279)	(279)	(86)	(280)	(279)
Cholesteryl ester	27.7	37.3	30.2	15.4	13.6
Free cholesterol	10.2	12.1	14.2	3.6	4.9
Triglyceride	46.2	13.1	10.8	3.4	10.9
Phospholipid	6.5	12.6	21.3	28.8	20.5
Protein	9.2	24.7	23.5	48.8	49.9

[a] Fractions were isolated by sequential ultracentrifugation.

Residue number:		(1)	(5)	(10)	(15)	(20)	(25)	(30)	(34)
N-terminal sequence.	(3) Man:	Asp-Glu-Pro-	Pro-Gln-Ser-	Pro-Trp-Asp-Arg-	Val-Lys-Asp-Leu-	Ala-Thr-Val-Tyr-	Val-Asp-Val-	Leu-Lys-Asp-Ser-	Gly-Arg-Asp-Tyr-Val-Ser-Gln-Phe-Gln-
	(138) Chicken:	Asp-Glu-Pro-	-----Gln-	Pro-Glu-Leu-					
	(120) Turkey:	Asp-Asp-Asn-	-----Gln-	Thr-Pro-Leu-Asn-Glu-	Ile-				
	(206) Dog:	Asp-Glu-Pro-	-----Gln-	Ser- Pro-Trp-Asp-Arg-	Val-Lys-Asp-Leu-	Ala-Thr-Val-Tyr-	Val-Asp-Ala-	Val- Lys-Asp-Ser-	Gly-Arg-Asp-Tyr-Val-Ala-Gln-Phe-Glx-
	(203) Dog:	Asp-Glu-Pro-	-----Gln-	Ser- Pro- Trp-Asp-					
	(222) Rabbit:	Asp-Glu-Pro-	-----Arg-Ser-	Ser- Trp-Asp-Lys-	Ile- Lys-Asp-Phe-	Ala-Thr-Val-Tyr-	Val-Asx-Thr-Val-	? -Asx-	? -Gly-Glu-Glu-Tyr-Val-Ala-Leu-
	(184) Swine:	Asp-Asp-Pro-	-----Gln-	Ser- Pro- Trp-Asp-Arg-	Val-				
	(147) Patas monkey:	Asp-Glu-Pro-	Pro-Gln-	Thr-Pro-Trp-Asp-Arg-	Val-Lys-Asp-Leu-	Val-Thr-Val-Tyr-	Val-Glu-		
	(280) Baboon:	Asp-Glu-Pro-	Pro-Gln-	Ser- Pro-Trp-Asp-Arg-	Val-Leu-Val-Thr-	Val-Tyr-Val-Asp-	Ala- Leu-Lys-Asp-	Ser-Gly-Arg-Asp-Tyr-Val-	
	(281) Chimpanzee:	Asp-Glu-Pro-	Pro-						
C-terminal sequence.	(3) Man:	-Leu-Asn-Thr-Gln.							
	(138) Chicken:	-Leu-Val-Ala-Gln.							
	(120) Turkey:	-Leu-Val-Thr-Ala.							
	(206) Dog:	-Lys-Leu-Ala.							
	(203) Dog:	-Leu-Ala-Gln.							
	(222) Rabbit:	-Thr-Leu-Gln.							
	(258) Pig:	-Leu-Gln.							
	(250) Pig:	-Lys-Thr-Ala-Leu.							
	(270) Rhesus monkey:	-Thr-Gln.							
	(280) Baboon:	-Leu-Ser-Thr-Gln.							
	(281) Chimpanzee:	-Thr-Gln.							

Fig. 5. Comparison of the amino- and carboxyl-terminal sequences of avian, canine, rabbit, porcine, nonhuman primate, and human apo A-I. The numbering of residues is based on the sequence of human apo A-I (3). Residue 4 in the N-terminal sequence of avian, canine, rabbit, and swine A-I has been placed in apposition to residue 5 of the human protein in order to demonstrate homology. References from which the data were taken are shown in parentheses.

suggested to Mahley and colleagues (147) that LDL-II was equivalent to the HDL_1 fraction seen in other species (e.g., dog (142), rat (163), and swine (143)), while LDL-I appeared to correspond to LDL itself. However, dog, rat, and swine HDL_1 lack detectable B apoprotein. Further study of the monkey LDL-II fraction showed that it cross-reacted immunochemically with antiserum to human Lp(a), and indeed it appears that to date, the Patas monkey is unique among the primates in possessing this lipoprotein.

HDL was the principal lipoprotein class, and resembled human HDL in composition and in particle diameter (70–100 Å). The major apolipoproteins showed a marked degree of homology with human apo A-I and apo A-II, although the A-II apoprotein existed as a monomer. An interesting variation in the amino-terminal sequence of apo A-I was the presence of proline residues at positions 3 and 4 of the Patas and human proteins, as compared to the avian, canine, rabbit, and porcine proteins which uniformly lack the second proline residue. Such a deletion throws the sequences of the A-I of the latter species out of phase by one amino acid with respect to the human polypeptide (**Fig. 5**). The possession of a second proline residue at position 4 in Patas apo A-I is until now a characteristic shared only by other Old World monkeys, i.e., the baboon (280) and chimpanzee (281). Fig. 5 reveals several points of note. Thus the occurrence of N-terminal aspartic acid in apo A-I is invariant, extending also to the rat polypeptide (139). Moreover, a high degree of homology is apparent in the N-terminal sequence of apo A-I in these diverse mammalian species. By contrast, the C-terminal sequences vary markedly, even to the extent that disparate results have been reported for the canine and porcine proteins. The species may however be tentatively divided into two groups on the basis of the carboxyl-terminal residues, i.e., the turkey (120), rat (139), and possibly dog (206) with C-terminal alanine, while the second group, namely chicken (138), rabbit (222), possibly pig (258), rhesus monkey (270), baboon (280), and chimpanzee (281) share C-terminal glutamine with the human apolipoprotein. Whether, as these observations might suggest, the N-terminal portion of apo A-I plays a common functional role in stabilizing the structure of mammalian HDL through lipid-binding, and whether its C-terminal portion is more species-specific and related to antigenic expression, remains indeterminate.

In addition, to the possession of analogues to the human A-I, A-II, and B apolipoproteins, the Patas monkey also has an apo E analogue, as demonstrated by the isolation of such a polypeptide from the β-migrating VLDL of cholesterol-fed animals (185). The African green monkey (*Cercopithecus aethiops*), of the

vervet subspecies, similarly possesses a polypeptide analogous to human apo E (282). The apolipoprotein was not however among the six isolated and characterized from the major vervet HDL fraction by Parks and Rudel (282), but rather was present in a minor HDL subfraction of higher molecular weight (282). The major protein component of vervet apo HDL could be purified by gel filtration chromatography in urea, and represented ca. 70% of the total apoprotein: it is a counterpart to human apo A-I, resembling the human protein in its molecular weight (28,000), polymorphism, migration in alkaline polyacrylamide gels containing urea, and amino acid composition (281). The second most abundant HDL apolipoprotein (ca. 10% of the total) was homologous with human apo A-II, although like the A-II polypeptide of other monkeys (rhesus (270) and baboon (280)), it exists in monomeric form and has a molecular weight of 8,500. This protein was isolated by ion-exchange column chromatography and purified by preparative isoelectric focusing. A similar approach was employed for the four additional low molecular weight polypeptides present in minor amounts in vervet apo HDL. These represented counterparts to the human C-III, C-II, and (two) threonine-poor apolipoproteins (282, 283). Since the latter components may on occasion account for up to 60% of vervet apo HDL, this Old World primate appears to provide a unique experimental model for the investigation of the function and metabolism of these drug-induced polypeptides (283).

Apes. The chimpanzee, (family Pongidae, genus Pan), together with the higher apes, i.e., the gorilla and orangutan, are the species which occupy the position closest to man on the phylogenetic scale. As such, the chimpanzee has LDL levels (338 mg/dl serum) approaching those generally seen in man (~350–400 mg/dl), although HDL is the dominant class. Moreover, the preponderance of the HDL_2 subfraction, so typical of the other Old World monkeys examined until now, is also apparent in the chimpanzee (278, 284). Variable VLDL levels (40–104 mg/dl) have been detected (284, 285). Proportions of sphingomyelin in chimpanzee HDL phospholipids were superior to those found in baboon and rhesus HDL, but did not attain the levels characteristic of human HDL (284). Fatty acid analysis revealed an overall resemblance in the lipoprotein lipid esters in corresponding human and chimpanzee fractions (284, 285).

The most striking aspect of chimpanzee lipoproteins is the presence in the protein moiety of the HDL of an apo A-II homologue in dimeric form (281, 284); a counterpart to human apo A-I was also present (281, 284). Chimpanzee apo A-I and apo A-II were comparable to the human polypeptides in molecular weight, as well as in amino- and carboxyl-terminal amino acids; the chimpanzee apolipoproteins cross-reacted with antiserum to the corresponding human fractions. Despite such high degrees of homology (which included the presence of a cysteine residue at position 6 of chimpanzee apo A-II and the microheterogeneity of its apo A-I), minor disparities have been detected in their amino acid compositions (281, 284). The relevance of the dimeric form of apo A-II to the role of this apolipoprotein in lipid binding and the stability of the HDL particle in man and chimpanzee remains unclear.

New World Monkeys. The New World monkeys are members of the family Cebidae, which comprises the genera Saimiri (as exemplified by the squirrel monkey, *Saimiri sciureus*), Cebus (the capuchin monkey, e.g., *Cebus albifrons*), and Ateles (the spider monkey, *Ateles geoffroyi*). While this group is more distantly related taxonomically to man than members of the family Cercopithecoidea, the scant information available indicates that the profile and structural characteristics of the New World monkey lipoproteins do not differ to a significantly greater extent from those of man than do those of the Old World species (see ref. 145). While Calvert (140) considered aspects of the response of the various New World genera to dietary lipid and cholesterol, Chapman, McTaggart, and Goldstein (145), in the course of a recent investigation of the serum lipoproteins of the common marmoset (*Callithrix jacchus*), have discussed comparative facets of the lipoprotein profile in New World primates. Briefly, the levels of LDL found in the marmoset, squirrel monkey (286), and spider monkey (287), on low-fat control diets, approach those of man more closely than in several species of Old World monkey (86, 278, 287). However, a number of New and Old World primates share the characteristics of transporting about one-half of their total cholesterol in HDL. Furthermore, the principal form of HDL tends to be HDL_2, rather than HDL_3 as in man. Such distinctions are probably manifestations of fundamental differences in the regulation of lipoprotein formation, metabolism, and degradation in these primates. At the same time, the high concentrations of HDL_2 in certain monkeys may provide us with experimental models in which to probe some of the factors regulating the circulating levels of a class of lipoprotein increasingly implicated as a protective factor in atherogenesis (288).

CONCLUSIONS

The nature of the present review has been essentially descriptive; nonetheless, it has revealed some surprising inconsistencies in published data on the

serum lipoproteins of several animals (e.g., the rat). On the other hand, it has perhaps underlined the fact that our knowledge of lipid transport systems in other species is, at most, fragmentary.

Some general conclusions are in order. Thus, the structure of vertebrate lipoproteins appears to conform in large part to a two-compartment model, as propounded by Shen et al. (52). This suggests the sequestration of hydrophobic lipids (cholesteryl esters and glycerides) in a central apolar core with a surrounding shell consisting of the more polar components (protein, phospholipid, and cholesterol). Do the hydrocarbons and monoalkyl diacylglyceryl ethers of cyclostome and fish lipoproteins partition into the core however? These and other subtleties of the structural organization of animal serum lipoproteins await evaluation. In the course of the present treatise, it will have become apparent that analogues of two major human apolipoproteins, i.e., A-I and B, are widely distributed in the HDL and in the LDL, respectively, of vertebrate lipoproteins. Why should this be so? Is the high degree of conservation of apo A-I a result of its structural role in the HDL particle, or a reflection of its function as an essential cofactor for the esterification of HDL cholesterol by LCAT? Is the similar high conservation of apo B throughout animal phylogeny (80) a consequence of its fundamental structural role in the LDL particle, or of the specificity of the cellular LDL receptor (289) and of the role of this macromolecule in the delivery of cholesterol to cells? The answers to these important questions should be forthcoming in the next decade.

Finally, it is to be hoped that those researchers who are primarily concerned with the metabolic aspects of lipoprotein systems may integrate the structural information provided herein. Efforts should thus be made to evaluate the metabolism of a single molecular species of lipoprotein wherever possible; an example would be lipoprotein B (Lp-B) in the pig (247), which is homogeneous with respect to its protein moiety (i.e., apo B). Furthermore, the present discussion of the comparative aspects of serum lipoprotein profile and structure in animals may have revealed species in which a distinct and specific anomaly or perturbation occurs and whose study might further our basic knowledge of lipoprotein metabolism.

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