FUNCTIONS AND INTERRELATIONSHIPS OF DIFFERENT CLASSES OF PLASMA LIPOPROTEINS

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Lipids and Their Functions in Human Physiology.—Plasma lipoproteins transport the biologically important lipid compounds: triglycerides, phospholipids, cholesterol, and cholesteryl esters. Insight into the function of plasma lipoproteins may be obtained, in part, from available information on the physiologic functions of these major classes of lipids. Briefly these functions include the following:

(*a*) Triglycerides are a source of fatty acids. Fatty acids can be oxidized by tissues for energy or stored as an energy reserve in triglyceride form. In response to energy needs, the storage triglycerides are hydrolyzed and the unesterified fatty acids are released into the circulation for transport to tissues.

(*b*) Phospholipids are a major structural unit of many cellular membranes, which include plasma membranes as well as membranes of cytoplasmic organelles, such as mitochondria and microsomes. These lipids concentrate at polar-apolar interfaces and stabilize lipoprotein structures in aqueous media.

(*c*) Unesterified cholesterol is a component of many cell membranes and also serves as a precursor in the formation of bile acids and steroid hormones.

(*d*) Cholesteryl esters are present chiefly in the liver, adrenals, and plasma. Their functional role is still unclear.

Based on the above functions of the major lipids in man, lipoproteins rich in triglycerides would be expected to participate in some transport phase of energy metabolism, while those rich in cholesterol and cholesteryl esters might be expected to have some transport function in sterol metabolism. One function of the phospholipids and proteins of the lipoproteins would be the stabilization of the highly apolar lipids (triglycerides, cholesterol, and cholesteryl esters) in plasma.

Major Classes of Plasma Lipoproteins and Their Lipid Composition.—Triglyceride-rich lipoproteins: Chylomicrons and very low density lipoproteins (VLDL):[1, 2] Chylomicrons contain approximately 98 per cent lipid and 2 per cent protein; triglycerides are the predominant lipid (89%), while phospholipid and cholesterol contents are 4 and 3 per cent, respectively. Cholesteryl ester content is 4 per cent. VLDL contain approximately 88–95 per cent lipid and 5–12 per cent protein; triglyceride, phospholipid, and cholesteryl ester contents are 50–60 per cent, 20–25 per cent, and 18–26 per cent of the lipid moiety, respectively.

The above chemical composition data indicate that the chylomicrons and VLDL are involved in some phase of energy metabolism because of their high content of triglyceride.

Cholesteryl ester and phospholipid-rich lipoproteins: Low density lipoproteins (LDL) and high density lipoproteins (HDL):[1, 2] LDL contain approximately 78 per cent lipid and 22 per cent protein; cholesteryl ester, phospholipid, and triglyceride contents are 55, 30, and 8 per cent of the lipid moiety, respectively.

HDL contain approximately 50 per cent lipid and 50 per cent protein; phospholipid, cholesteryl ester, and triglyceride contents are 43, 45, and 6 per cent of the lipid moiety, respectively.

From their lipid composition, the functions of the LDL and HDL are not readily apparent. However, the relatively high contents of cholesteryl esters suggest some role in the transport and metabolism of these compounds.

Structural Properties of Plasma Lipoproteins.—The structural properties of plasma lipoproteins have been extensively investigated and are of interest in any evaluation of lipoprotein function in lipid transport. Electron microscopy has provided a direct visualization of lipoproteins with apparently minimal introduction of structural artifacts.[3–6] Figure 1 shows representative micrographs of the four major classes of lipoproteins.

Chylomicrons appear as spherical particles which range in diameter from approximately 1,200–11,000 Å. However, in plasma obtained after fat ingestion, the bulk of the triglyceride was found in chylomicron particles of 1,500 to 4,000 Å diameter.[7] No indication of any specific surface organization is apparent in these preparations fixed with osmium tetroxide. Electron micrographs of sections of embedded chylomicrons which were previously exposed to osmium tetroxide show a dense outer layer of material.[8] Analysis of an outer layer of material isolated from chemically untreated particles showed it to consist primarily of protein, phospholipid, and cholesterol.[9] These particles provide a large surface for interaction with enzymes and cellular surfaces.

Osmium tetroxide-fixed very low density lipoproteins are seen as spherical macromolecules with diameters ranging in size from 300 to 700 Å. No distinct surface features can be discerned by the procedures used.

Low density lipoproteins appear primarily as spherical molecules with a mean diameter of approximately 216 Å (range 170–260 Å). Upon contact with each other the LDL show considerable distortion and nonuniformity in shape. Surface features appear regular; however, regions of low electron density appear to bridge adjacent molecules. No specific subunit structure is apparent from these micrographs.

Free-standing high-density lipoproteins show structures with a mean diameter of 86 Å (range 74–98 Å), frequently with an electron dense region in the center. The images suggest a structure composed of subunits aggregating to form an intact macromolecule. The number of apparent subunits in such structures varies. The subunit structure of the HDL and the relative lability of the macromolecule may have substantial implications to the function of these lipoproteins.

Concentrations of Lipoproteins in Human Plasma.—Studies of plasma lipoprotein levels in samples of the American population are impressive in showing a great diversity of lipoprotein distributions. These studies also indicate the presence of several statistically significant relationships among specific classes of lipoproteins. Representative levels of lipoproteins in human plasma obtained by ultracentrifugal procedures[1, 10] are shown in Table 1.

An outstanding feature of these data is the considerable difference between males and females in the distribution of plasma lipoproteins. On the average,

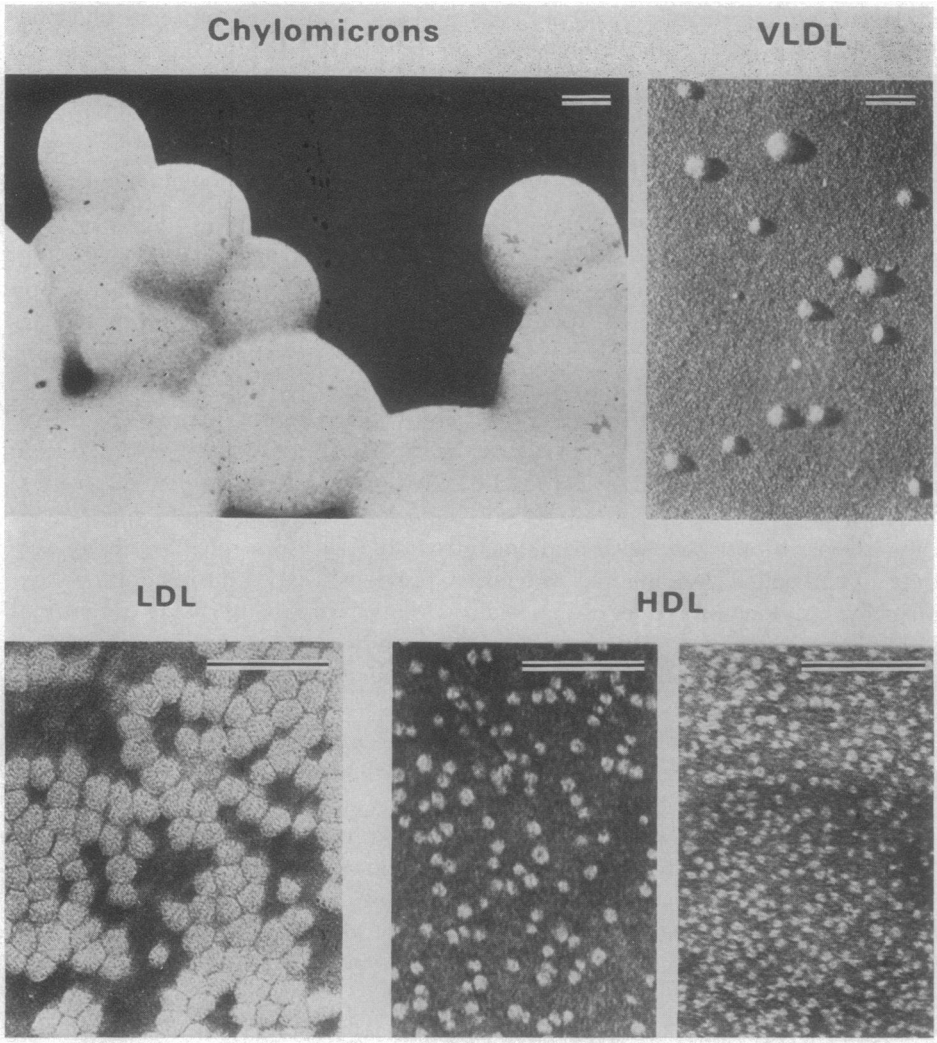

FIG. 1.—Electron micrographs of plasma lipoproteins. Upper micrographs show chylomicrons and VLDL after fixation in buffered osmium tetroxide and shadowing with a platinum-palladium-gold alloy. Lower micrographs show LDL and HDL (subclasses HDL_2 and HDL_3, *left* to *right*, respectively) after negative staining with sodium phosphotungstate. All reference markers designate 0.1 μ.

males (in this age range) show higher levels of VLDL and LDL and lower values of HDL than females. The difference in HDL concentrations between males and females results primarily from the presence of higher levels of HDL_2 (a subclass of the HDL) in females than in males. These data also suggest an inverse relationship between the VLDL and LDL, and the HDL.

Relationship of Plasma Lipoprotein Levels to Age.—LDL levels increase with age in males and females, especially in the third decade of life.[11] In males, the

TABLE 1. *Plasma lipoprotein concentrations.*

	Chylomicrons	VLDL	LDL	HDL
Males	12 ± 13	129 ± 122	439 ± 99	300 ± 83
Females	2 ± 3	59 ± 63	389 ± 79	457 ± 115

These values were obtained from 16 males and 16 females in health (35–50 years). Mean values and their standard deviations are given in mg/100 ml.

The above values for lipoprotein levels were obtained from ultracentrifugal studies performed on human serum rather than plasma. Since no significant differences in lipoprotein levels or properties between serum and plasma have been observed, the term "plasma lipoproteins" in this and other instances, where serum was studied instead of plasma, will be used throughout the text for uniformity of presentation.

rate of increase of mean LDL levels declines during the fourth decade, and after peaking, at approximately 45 years of age, the levels decrease. In females, the peak values of LDL are attained by approximately 55 years. Increases in VLDL levels with age are more pronounced in males than in females. In males, the mean VLDL levels show a marked increase during the third and fourth decades. The peak occurs at approximately the fifth decade. Females show a gradual upward trend during this period at substantially lower values of VLDL concentrations. No significant age trends are observed for the HDL in either sex. The underlying bases for the above age statistical trends and their functional significance are not clearly understood.

Plasma Lipoprotein Relationships from Population Studies.—A regression curve of LDL on VLDL levels in the plasma of two large samples of human males (ages 30–39 and 40–49 years) shows a steady rise in LDL levels with increasing levels of VLDL.[11] At very high levels of VLDL, the curve slopes downward and the relationship between these two classes becomes inverse rather than direct. Furthermore, both VLDL and LDL levels show an inverse relationship with HDL_2 levels in the plasma of males. A modest inverse relationship is observed between VLDL and HDL_3 levels. Elevated levels of HDL_1 are frequently associated with very high concentrations of VLDL.[2] These statistical analyses indicate functional associations among the major classes of lipoproteins which apparently are related to the status of triglyceride metabolism.

In the plasma of males, a significant inverse relationship between the ultracentrifugal $S_f°$* rate of the major peak of the LDL and the level of VLDL has been observed.[10] An inverse relationship between the $S_f°$ rate of the major LDL peak and the total level of VLDL plus LDL has also been reported.[12] Since *in vitro* incubation of plasma high in VLDL increases the $S_f°$ rate of the major LDL peak, the basis for the above inverse relationship *in vivo* is apparently not a result of physical-chemical interaction of LDL with large amounts of VLDL.[13] The functional significance of this relationship has not yet been explained.

Additional evidences of the significant influence of elevated plasma triglycerides *in vivo* on lipoprotein properties is the observation of a strong direct relationship between plasma levels of VLDL and the percentage content of triglyceride in the HDL.[10] A modest relationship between plasma VLDL levels and the percentage content of triglyceride in the LDL is also observed. *In vitro* incubation of plasma with high levels of VLDL results in a significant increase in the triglyceride content of the HDL and LDL fractions.[14]

The above relationships all point to a strong impact of triglyceride metabolism

and transport on the concentrations and compositions of the LDL and HDL. In subsequent sections, chemical and metabolic relationships among these classes of lipoproteins will be described which will help clarify some of the above associations.

Chemical Interrelationships among Plasma Lipoproteins.—Protein moiety: Extensive immunochemical studies have established that normally occurring chylomicrons and VLDL contain within their protein moieties proteins or peptides which are also found separately in the LDL and HDL.[15] Thus, it appears that there is a most fundamental chemical interrelationship among the triglyceride-rich lipoproteins and the LDL and HDL which results from their mutual utilization of some specific carrier proteins or peptides for lipid transport. The immunochemically different protein moieties of the intact LDL and HDL have been designated apoLDL and apoHDL. Hence, the chylomicron and VLDL classes contain apoLDL and apoHDL as part of their protein moieties. More recently, however, there has been considerable progress in resolving different subunit peptides within the apoprotein moieties of the major lipoprotein classes.[16, 17] (A discussion of these subunit peptides is presented in another section of this symposium.) Specific interrelationships among the plasma lipoproteins based on the distribution of such subunit peptides are also indicated by these studies.

Lipid moiety: Certain lipids of the plasma lipoproteins are interrelated in the sense that they are in continual dynamic exchange among the various lipoproteins. In particular, lecithin, cholesterol, and triglyceride moieties have been reported to undergo extensive exchange *in vivo* and *in vitro* apparently during collisions of the lipoproteins in plasma.[18–20] Cholesteryl esters have not been observed to exchange among the lipoprotein classes.[21] However, cholesteryl esters of the LDL and HDL have been found to engage in reciprocal transfer with VLDL triglycerides during *in vitro* incubation of plasma rich in VLDL.[14]

Functions of Chylomicrons and VLDL.—In the transport of fatty acids to tissues for energy production or storage, two chemical forms of fatty acids are found in plasma.[15, 22] One form is the esterified or triglyceride form, which is transported primarily by the chylomicrons and VLDL; the other form is the unesterified form, which is transported by plasma albumin. Triglycerides transported by chylomicrons and by some VLDL are of exogenous (dietary) origin. Dietary fatty acids transported by the chylomicrons from the intestine are subsequently found in the liver (where they may undergo oxidation or incorporation into liver triglycerides and storage, or secretion into plasma as VLDL), adipose tissue (where they are stored as triglycerides and are available for subsequent hydrolysis, secretion, and transport in plasma as albumin-bound unesterified fatty acids), and other tissues (where they are usually oxidized). Chylomicrons rapidly appear in plasma after fat ingestion and, when injected *in vivo*, have been found to clear from plasma with a half time of approximately 10–15 minutes.

Triglycerides transported by VLDL are primarily of endogenous origin, formed mainly in the liver from carbohydrates or unesterified fatty acids. The formation and secretion of VLDL may occur in various nutritional states.[23] In the

fed state, with ample carbohydrate, VLDL secretion by the liver occurs following synthesis of triglycerides from carbohydrates. In the fasted state, secretion of VLDL by the liver may occur following synthesis of triglyceride using unesterified fatty acids mobilized from adipose tissue in response to the oxidative needs of the various tissues. The fatty acids of the triglycerides transported by VLDL may be utilized or stored in tissues.

Metabolism of Chylomicrons and VLDL and its Relationship to LDL and HDL Levels in Plasma.—A complete picture of the various dynamic events occurring during the removal from plasma of the triglycerides of chylomicrons and VLDL by the tissues is not available. However, there is abundant evidence that a hydrolytic process is probably involved in some phase of this removal.[24] The enzymes probably catalyzing this hydrolysis have been detected in plasma after parenteral administration of heparin.[25] Normally, these enzymes are presumed to be located at or near the capillary wall. During the acute hydrolysis of plasma triglycerides which follows heparin administration, there is rapid degradation of chylomicrons and VLDL into smaller lipoprotein products which have ultracentrifugal flotation properties similar to LDL and HDL.[26] It is possible that during physiologic removal of chylomicrons and VLDL triglycerides, a comparable but slower degradation occurs and the lipoprotein products so formed may be a source of normal plasma LDL and, possibly, HDL. After *in vivo* heparin administration, the chemical properties of the LDL products are comparable to those of the normally occurring LDL. However, the HDL products contain appreciable phospholipids and less of the other lipid components normally occurring in HDL.[27, 28] The lipid composition of these HDL products may be normalized during their circulation in plasma to that of the regular HDL by the activity of the plasma enzyme, lecithin:cholesterol acyltransferase. This enzyme catalyzes a transesterification reaction between lecithin and cholesterol in plasma and is particularly reactive with HDL lipids.[29] Additional evidence suggestive of a precursor-product relationship between the VLDL and LDL is provided by tracer studies *in vivo* using protein-labeled lipoproteins,[30, 31] and from studies evaluating changes in lipoprotein distributions during metabolic (fasting of hypertriglyceridemic subjects[32]) and pharmacologic (administration of ethyl-*p*-chlorophenoxyisobutyrate to hypertriglyceridemic subjects,[33] or insulin to subjects in diabetic acidosis[34]) situations where substantial shifts in plasma lipoprotein distributions occur. These shifts are characterized by marked decreases in VLDL and increases in LDL (usually) and in HDL (occasionally) levels.

Function of ApoLDL or LDL in the Transport of Triglycerides by Chylomicrons and VLDL.—Based on several lines of evidence, the synthesis and appropriate availability of apoLDL or LDL appear to be crucial requirements for the formation and secretion of chylomicrons and VLDL. Inhibition of protein synthesis in the intestine of rats results in lipid accumulation in the mucosal cells after a fatty meal and the curtailment of chylomicron formation and secretion.[35] Inhibition of the liver's capability for synthesis or secretion of LDL, by administration of orotic acid to rats, leads to triglyceride accumulation in the liver and in-

hibition of VLDL formation and secretion.[36, 37] With orotic acid, protein synthesis is not inhibited and HDL are still secreted by the liver. In the human genetic disorder, abetalipoproteinemia, where plasma LDL are absent, transport of triglyceride from the intestine in chylomicron form and from the liver in VLDL form is also absent.[15] In this disorder, lipid accumulation in the intestinal mucosal cells also occurs after fat ingestion. The above observations, in aggregate, suggest quite strongly that apoLDL (or LDL) is probably a crucial factor in effecting the formation and secretion of chylomicrons and some VLDL by the intestine and VLDL by the liver.

Functions of ApoHDL and HDL in Chylomicron and VLDL Metabolism.—Although apoHDL is consistently detected in chylomicrons and VLDL, it is not required for the initial formation and secretion of these lipoprotein classes.[15] There is considerable evidence that chylomicrons and VLDL probably pick up some apoHDL or HDL upon entry into the bloodstream. Uptake of apoHDL or HDL by rat lymph chylomicrons occurs during *in vitro* incubation of these chylomicrons with isolated HDL.[38] In the course of these incubations, some phospholipid is transferred from the VLDL to the HDL, and some cholesterol is transferred from the HDL to the VLDL.

The functional significance of the apoHDL or HDL on chylomicrons and VLDL is not altogether clear. However, *in vitro* studies have shown that HDL or apoHDL-phospholipid complexes can activate enzymes (lipoprotein lipase[39] and possibly lecithin:cholesterol acyltransferase[29]) whose substrates are lipoprotein lipids. Furthermore, in subjects with a deficiency in plasma HDL (Tangier disease) triglyceride removal is less efficient than in normal subjects.[15]

Other Functions of Plasma Lipoproteins.—Clearly, a major function of the constituents (apoproteins and possibly lipids) of the LDL and HDL appears to be the transport of the energy-rich triglycerides. Triglycerides are highly apolar compounds and for transport in aqueous media require appropriately hydrophilic materials, such as the apoproteins and phospholipids. Plasma lipoproteins also transport considerable amounts of another class of apolar lipids (cholesteryl esters) which have solubility properties comparable to those of the triglycerides. The HDL and particularly the LDL could be considered transport vehicles for these lipids in plasma. However, unlike triglycerides, the function of cholesteryl esters is not clear and, hence, the purpose of their transport is little understood. Cholesteryl esters are present in tissues actively utilizing cholesterol as a metabolic precursor, and it has been suggested that they may function as reservoirs of readily available cholesterol.[40]

Recent studies suggest a possible role for plasma HDL in the transport as well as in the metabolism of cholesteryl esters.[29] In the genetic HDL-deficiency state, Tangier disease (homozygous form), extensive accumulation of cholesteryl esters occurs in the reticuloendothelial tissues.[41] In another disease state characterized by reduced levels of plasma HDL, cholesteryl esters do not accumulate in these tissues; however, in this disease, plasma cholesteryl ester levels are abnormally low owing to a deficiency in the plasma enzyme lecithin:cholesterol acyltransferase.[42] The cholesterol-esterifying reaction catalyzed by

this enzyme has been proposed as the major source of cholesteryl esters in human plasma.[29] Furthermore, several reports indicate that the preferred lipoprotein substrate for lecithin:cholesterol acyltransferase is HDL and that the HDL surface is the probable locus for the transesterification reaction. In Tangier disease, in which plasma lecithin:cholesterol acyltransferase activity has been detected, it is possible that the site of production and the disposition of the product cholesteryl esters may be abnormal and lead to the accumulations noted above. Another possibility is that the HDL may be involved in the transport of cholesteryl esters from the reticuloendothelial tissues to the liver for catabolism, and hence a deficiency in HDL would result in an accumulation of cholesteryl esters in these tissues.[43]

The lecithin:cholesterol acyltransferase reaction with HDL may also play some role in processes regulating the cholesterol content of human red blood cells.[29] In lecithin:cholesterol acyltransferase deficiency, the esterification of lipoprotein cholesterol does not occur and as a result the predominant sterol in plasma is unesterified cholesterol. In this situation, the cholesterol content of the membranes of the red cells is abnormally elevated and the percentage composition of their phospholipids is grossly different from normal cells.[44] Furthermore, the morphological features of such cells are markedly different from normal cells. *In vitro* incubation studies with normal whole blood have shown a significant reduction in the cholesterol content of red cells during the esterification of lipoprotein cholesterol by lecithin:cholesterol acyltransferase.[45] Apparently the red cells become depleted of their normal charge of cholesterol by transferring some of their cholesterol to lipoproteins, whose cholesterol has undergone transesterification to cholesteryl esters by lecithin:cholesterol transferase activity. These observations indicate a possible process of cholesterol removal, involving the acyltransferase, which may be operative *in vivo* in controlling the cholesterol content of red blood cells. The accumulation of cholesterol in the red cells of lecithin:cholesterol acyltransferase-deficient subjects may be due, in great part, to a lack of such a removal process.[44]

Plasma Lipoproteins in Other Species.—In conclusion, it is of interest to note the occurrence and diversity of plasma lipoprotein distributions in marine and other terrestrial mammals. Plasma lipoproteins in these mammals presumably serve transport functions comparable to those in humans. However, the specific types of apoproteins and their distribution among the plasma lipoproteins may differ considerably from those in humans.[36] Further, the dietary patterns of terrestrial and marine mammals in the wild are markedly different, and probably place different demands on lipoprotein metabolism with respect to triglyceride and, possibly, cholesteryl ester transport in the bloodstream.

Carnivores, such as the dog, dolphin, porpoise, and seal, generally exhibit lipoprotein distributions very high in HDL and low in LDL.[46, 47] Some herbivores, such as the bison,[48] cow,[49] and rabbit[50] also show lipoprotein distributions with HDL as the predominant lipoprotein class. Other herbivores, such as the guinea pig,[46, 51] and omnivores, such as man and baboon,[46] show plasma lipoprotein distributions with considerably higher percentages of LDL than the carnivores. These comparative observations are clearly indicative of a general utilization of

lipoprotein structures, by mammals, for effecting the transport of lipids in plasma. Our complete understanding of the underlying metabolic bases responsible for the observed diversity of plasma lipoprotein distributions awaits further investigation.

Summary.—A major function of plasma lipoproteins is in the transport of exogenous and endogenous triglycerides. Many of the prominent interrelationships among the plasma lipoproteins reflect this important function. Recent studies indicate a possible role for certain plasma lipoproteins in the regulation of the cholesterol and cholesteryl ester contents of cells and tissues. The full elucidation of the various functions of plasma lipoproteins remains a fascinating and basic problem in mammalian biology.

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* $S_f°$ values are flotation rates in Svedberg units for lipoproteins in a medium of d 1.063 gm/ml (NaCl, 26°, 52,640 rpm) and are rates corrected for concentration and Johnston-Ogston effects.

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