1985

Studies on Plasma Lipoprotein-Cholesterol Metabolism of the Rat Employing Cellulose Acetate Electrophoresis

Isaac Bruce Rosenzweig
Loyola University Chicago

STUDIES ON PLASMA LIPOPROTEIN-CHOLESTEROL METABOLISM OF THE RAT EMPLOYING CELLULOSE ACETATE ELECTROPHORESIS

by

Isaac Bruce Rosenzweig

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

April

1985

ACKNOWLEDGEMENTS

Dr. Joseph R. Davis my major advisor, and friend these past years has provided the support, training and guidance that have made this work possible. It has been a pleasure and a privelege to have worked with him and I look forward to a continued association in the future.

I would like to thank Dr. A. G. Karczmar, the faculty and the students of the department for their support, understanding and the invaluable lessons they have taught me. I would also like to thank my dissertation committee for kindly consenting to evaluate this work.

I would like to gratefully acknowledge the financial support of Loyola University and the Schmitt Dissertation Fellowship Committee which allowed me the means to continue my studies at Loyola.

I am deeply indebted to my friends and family who have helped me through these times and have offered their support and understanding as I was not able to see them as often as I would have liked.

More recently I have become indepted to Jack Corliss, Dave Gabrovitch and Dr. Peter Dordal for their help with the Loyola computer system.

VITA

The author Isaac Bruce Rosenzweig is the son of Leon E. and Florence (Bernstein) Rosenzweig. He was born in Rockford, Illinois on May 12, 1952, graduated from the Rockford public school system and enrolled at the University of Illinois and subsequently graduated in June of 1974 with a Bachelor of Sciences Degree in Biochemistry. He worked as a clinical technician for the Dept of Biochemistry at Michael Reese Hospital under the direction of Dr. Samuel Natelson beginning in 1974. He became an assistant lab manager of the Biochemistry department of Michael Reese Hospital in 1978 and began his graduate education at Loyola University of Chicago Stritch School of Medicine in the Department of Pharmacology in July of 1978.

While a graduate student at Loyola the author was awarded a Loyola University of Chicago Basic Science Fellow (1980-1983) and a Schmitt Dissertation Fellowship (1984-1985).

The author has been a member of the American Chemical Society since 1979 and a member of the American Association for the Advancement of Science since 1982. He is the co-author of seven scientific publications.

PUBLICATIONS AND ABSTRACTS

Davis, Joseph R. and Rosenzweig, I. Bruce, Electrophoretic separation of a novel prealbumin lipoprotein (PAL) from normal rat serum. Fed. Proc. 38: 550, 1979.

Davis, Joseph R., Davis, Ann R. and Rosenzweig, I. Bruce, Prealbumin lipoprotein (PAL): A high density lipoprotein of rat plasma separated by cellulose acetate electrophoresis. Fed. Proc. 41: 718, 1982.

Rosenzweig, I. Bruce, Davis, Ann R. and Davis, Joseph R. In vitro transfer of high density lipoprotein cholesterol from alpha$_1$ lipoprotein to prealbumin lipoprotein in the rat. Fed. Proc. 41: 1622, 1982.

Rosenzweig, I. Bruce and Davis, Joseph R., p-Chloromercuriphenylsulfonic acid (PCMPS) inhibition of in vitro transfer of high density lipoprotein-cholesterol from alpha$_1$ lipoprotein to prealbumin lipoprotein in rat plasma. Fed. Proc. 42: 1059, 1983.

Rosenzweig, I. Bruce and Davis, Joseph R., In vitro formation of plasma prealbumin lipoprotein (PAL) from alpha$_1$ lipoprotein (α_1L) catabolism in adult male rats following clearance of PAL by in vivo administration of ethinyl estradiol. Fed. Proc. 43: 482, 1984.

Davis, Joseph R., Davis, Ann R. and Rosenzweig, I. Bruce, A comparison of the cellulose acetate electrophoretic serum lipoprotein-cholesterol profile of the adult male rat with other species. Atherosclerosis (Submitted for publication).

Rosenzweig, I. Bruce, Davis, Ann R. and Davis, Joseph R., Plasma clearance and intravascular formation of prealbumin lipoprotein from alpha$_1$ lipoprotein catabolism in the adult rat. Atherosclerosis (In preparation).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CONTENTS OF APPENDICES

LIST OF NONSTANDARD ABBREVIATIONS

α_1L	alpha$_1$ lipoprotein
α_2L	alpha$_2$ lipoprotein
βL	beta lipoprotein
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
EC	esterified cholesterol
HDL	high density lipoprotein
LCAT	lecithin:cholesterol acyltransferase
LDL	low density lipoprotein
LPL	lipoprotein lipase
PAL	prealbumin lipoprotein
PCMB	p-chloromercuribenzoate
PCMPS	p-chloromercuriphenylsulfonate
TC	total cholesterol
UC	unesterified cholesterol
VLDL	very low density lipoprotein

CHAPTER I

INTRODUCTION AND REVIEW OF THE RELATED LITERATURE

I. CHOLESTEROL

A. Cholesterol

1. Endogenous Sources of Cholesterol

Cholesterol shown in figure 1 is formed from two carbon units provided by acetyl CoA. Cholesterol synthesis begins by the condensation of two acetyl CoA molecules to acetoacetyl CoA, which is catalyzed by acetoacetyl CoA thiolase. The next step for cholesterol synthesis catalyzed by HMG CoA synthase involves the formation of 3-hydroxy-3-methyl glutaryl CoA from acetoacetyl CoA. Mevalonic acid is then produced by hydrolysis of the thiol ester bond which is catalyzed by the enzyme HMG CoA reductase. Mevalonic acid in a series of reactions forms farnesyl pyrophosphate a 15 carbon compound, which fuses with another molecule of farnesyl pyrophosphate to form squalene a process which is catalyzed by squalene synthetase. A hydroxyl group is added to the squalene molecule at the C-3 position in the beta stereochemical configuration by squalene oxidocyclase which also catalyzes the cyclization of squalene to lanosterol. Lanosterol after a few other modifications becomes the 27 carbon cholesterol with a hydroxyl group at the 3 beta position and a 5,6 carbon double bond (Bloch, 1965).

The rate determining step for cholesterol synthesis has been shown to be the conversion of β hydroxy-β methyl glutaryl coenzyme A to mevalonic acid and the regulatory enzyme responsible for this conversion is mevalonate:NADP oxidoreductase (acylating CoA), EC 1.1.1.34 (Nomenclature Committee, 1979) better known as HMG CoA reductase. Feeding rats high cholesterol diets has been shown to inhibit the conversion of β hydroxy-β methyl glutaryl coenzyme A to mevalonic acid (Frantz et al., 1954; Siperstein et al., 1960). In addition, a pronounced reduction in HMG CoA reductase activity was also noted when rats were fed cholesterol (Shefer et al., 1973). Bile acids appeared to be necessary for the decrease in HMG CoA reductase activity found in rat liver, but may only play a secondary role to that of cholesterol concentration. The mechanism of cholesterol's feedback regulation of HMG CoA reductase activity appears to be very complex since not only is activity, but also quantity of HMG CoA reductase affected by increasing sterol concentrations (Rodwell et al., 1976). A preference for absorbed cholesterol over that of endogenous production in the liver has been observed and postulated to be due to the more ready availability to the liver of chylomicron remnants containing cholesterol (Nervi et al., 1974). This may be true for other tissues as well, since extrahepatic tissue cholesterol synthesis appears to be regulated by cholesterol carried to the tissues from the blood by lipoproteins (see section II.C.4. LDL Catabolism). The precise mechanism for cholesterol feedback inhibition on HMG CoA reductase is as yet unknown.

Endogenous sources of cholesterol include almost every cell in the

animal, since virtually every tissue is capable of synthesizing cholesterol (Dietschy et al., 1967), yet the bulk of endogenous cholesterol of both human and rat is synthesized in the liver (Dietschy et al., 1971; Turley et al., 1981b). Studies have indicated that 50% of endogenous cholesterol in the rat is derived from the liver, 24% in the small bowel, 8% from the skin and 18% from the remaining tissues (Turley et al., 1981a). Unfortunately, cholesterol biosynthesis in rat tissues was determined using the digitonin precipitation method for unesterified sterols (Sperry, 1950), which was recently found to include methyl sterols that coprecipitated with the unesterified sterols. The rate of cholesterol biosynthesis determined using the digitonin precipitation technique may appear to be faster than what is actually occurring. The inaccuracy of the cholesterol biosynthesis determination would be due to the inclusion of methyl sterols as well as the desired unesterified sterol in the assay (Tabicik et al., 1983). Although the method for determining the rate and amount of cholesterol biosynthesis for each tissue may be of uncertain accuracy, the relative percents of cholesterol synthesis for each tissue, should be good rough estimates (Turley et al., 1981a). Moreover, despite the methodology used to determine tissue cholesterol biosynthesis it has been clearly demonstrated that the bulk of endogenous cholesterol synthesis occurs in the liver and small bowel of both rats and humans (Jeske et al., 1980).

Cholesterol is ubiquitous in the mammalian system, but certain tissues synthesize the cholesterol required for self maintenance while other tissues receive cholesterol requirements from circulating plasma

lipoproteins. The contribution of many rat tissues to their own cholesterol content and also the contribution of circulating lipoprotein cholesterol to tissue cholesterol content have been determined. Brain, ovary, skin, muscle and marrow were found to produce greater than 75% of their own cholesterol content. The adrenal produced only 4% of its own sterol content, the spleen 6%, the heart 28%, kidney 26% and lung 17%. Cholesterol not produced by the tissue is obtained from circulating plasma lipoproteins (Andersen et al., 1979; Turley et al., 1981a; 1981b).

2. Exogenous Sources of Cholesterol

Diet and bile are the two major sources of cholesterol in the intestine (Grundy, 1978). Cholesterol intake from an American diet has been found to average between 500-750 mg per day while biliary excretion of cholesterol averages between 750-1250 mg per day (Bennion et al., 1975; Grundy, 1972). Other sources of intestinal cholesterol include degraded intestinal cells and intestinal secretions, although their contributions to the total pool of cholesterol within the intestine have been difficult to assess (Miettinen et al., 1981). It has been determined that only 30-60% of cholesterol present in the human intestine is absorbed (Quintao et al., 1971). Moreover, the rat's ability to absorb cholesterol has also been demonstrated to be between 30-60% of the cholesterol present in the intestine (Cook et al., 1951; Pihl, 1955).

Cholesterol absorption is limited by its solubilization into micelles (Hofman et al., 1964) and only unesterified cholesterol can be

solubilized into these micelles, therefore cholesteryl esters present in the diet are hydrolyzed in the intestine and converted to unesterified cholesterol and free fatty acids by carboxylic-ester hydrolase, EC 3.1.1.13 (Nomenclature Committee, 1979), a nonspecific lipase secreted from the pancreas (Lombardo, et al., 1980). In addition, micelle formation has been shown to require conjugated bile acids and hydrolytic products of triglycerides, lecithin (phosphatidyl choline), fatty acids, monoglycerides and lysolecithin (Hofman et al., 1964). Therefore, cholesterol absorption has been found to be dependent on the intraluminal availability of bile acids and hydrolytic products of dietary fat (Pihl, 1955; Swell et al., 1955).

Cholesterol is absorbed by intestinal mucosal cells. Maximum absorption has been demonstrated to occur in the upper intestine where the micelles facilitate transport of cholesterol across the unstirred water layer adjacent to the surface of the luminal cell (Simmonds et al., 1967; Westergaard et al., 1976). Polar lipids necessary for cholesterol solubilization have also been found to be almost quantitatively absorbed at the proximal part of the small intestine (Simmonds et al., 1967), so cholesterol would precipitate within the lumen of the small intestine when the polar lipid concentration needed to solubilize cholesterol decreases (Simmonds et al., 1967), even though bile acids are mainly absorbed in the distil portion of the small intestine (Weiner et al., 1968). However, a high fat diet would allow the polar lipids to remain in the micelle longer, thus increasing cholesterol absorption. Cholesterol absorption into the intestinal cell is believed to occur

when the cholesterol containing micelle collides with the intestinal cell membrane and disaggregates following which bile salts and cholesterol diffuse across the membrane passively. The entire micelle would not be absorbed intact and those portions of the micelle not absorbed would be excreted in the feces (Chow et al., 1978; Schultz et al., 1971).

Intraluminal intestinal cholesterol is mostly unesterified, yet more esterified than unesterified cholesterol has been found in the intestinal lymph (Gallo et al., 1963). Microsomal acyl coenzyme A cholesterol acyltransferase, EC 2.3.1.21 (Nomenclature Committee, 1979), also known as ACAT, has been shown to be responsible for the formation of cholesteryl esters within the intestinal mucosal cell (Haugen et al., 1976). Unesterified cholesterol and cholesteryl esters have been found to be secreted into the mesenteric lymph after being incorporated into lipoproteins known as chylomicrons (Sabesin, 1976). Chylomicrons are then metabolized by an enzyme known as lipoprotein lipase into chylomicron remnants, which are taken up by the liver (Fielding et al., 1977). Chylomicron synthesis and catabolism will be covered in greater detail in a later section (see section II.C. Lipoprotein Synthesis and Metabolism).

B. Cholesterol Distribution

1. Membrane and Intracellular

Cholesterol has three major functions in mammalian organisms: (1) It is a required structural component of the plasma membrane of all cells. (2) It is the precursor for steroid hormones. (3) It is the precursor of bile acids (Havel et al., 1980). The unesterified cholesterol of tissues is found exclusively in the cell membrane, whereas excess intracellular unesterified cholesterol is esterified by ACAT and stored within the cell as a liquid cholesterol ester crystal (Brown et al., 1975c). Plasma cholesterol on the other hand, is found within substances known as lipoproteins.

2. Plasma Lipoprotein

Lipoproteins transport nonpolar lipids through the plasma. Lipoproteins are large macromolecular complexes which are pseudomicellar (Havel, 1975). Analysis of the correlations between size and chemical composition of lipoproteins in normal humans indicated lipoproteins are spherical, surrounded by a monolayer of cholesterol and phospholipids. Proteins appear to be closely packed with the hydrophilic head groups of phospholipids at the outer surface of the lipoprotein molecule. Hydrophobic ends of phospholipids and cholesterol surround and seem to be packed tightly at a liquid core of cholesteryl ester and triglyceride. The correlations between size and chemical composition of lipoproteins also indicated that sharply defined boundaries exist between the hydrophobic core and the amphipathic layer surrounding it. Proteins appear

to be at the same surface layer as the phospholipid head groups competing for that surface area. Also, the location of all unesterified cholesterol may be under the protein layer with the alcohol group in direct contact with protein and not the surrounding plasma water. Moreover, the analysis indicates that all lipoproteins regardless of size, have an identical structure. (Shen et al., 1977). However, studies using nuclear magnetic resonance suggested that unesterified cholesterol rapidly exchanges between the core and surface of lipoproteins (Lund-Katz et al., 1981). In addition, 40% of the lipoprotein unesterified cholesterol appeared to exist in the core of the molecule, while 60% appears to be present on the surface of the molecule associated with phospholipids. Moreover, the the nuclear magnetic resonance study implied that more than 90% of lipoprotein-unesterified cholesterol molecules belong to a single kinetic pool, from which they could be available for exchange with cholesterol molecules in cells or other lipoproteins (Lund-Katz et al., 1984).

C. Cholesterol Clearance

1. Bile and Sloughed Intestinal Cells

The main mode of cholesterol excretion has been demonstrated to be via the bile and is dependent on bile salt secretion (Hardison et. al., 1972; Wheeler, 1972). Unesterified cholesterol is dissolved within the bile and bile is formed by cholesterol catabolism, therefore cholesterol is excreted from the liver into the intestine in two forms. Moreover,

the bulk of the cholesterol which appears in the bile either in the form of bile acids or as unesterified cholesterol was found by one study to be derived from the liver of rats (Turley, et al., 1981b). However, it has been suggested that the major source of bile acids in man is from lipoprotein-cholesterol (Schwartz et al., 1981). Sloughed cells and secretions from the intestine as mentioned previously are also involved in cholesterol excretion, but it has been difficult to determine to what extent (Miettinen et al., 1981).

2. Skin

Skin surface sterols are a mixture derived both from local synthesis and from the plasma. Therefore, cholesterol has been found to be lost from the hair, desquamated cells, sweat and sebaceous secretions, which makes skin the second major source for cholesterol excretion in the human (Nikkari et al., 1974).

3. Urine

Studies on humans have shown that cholesterol is excreted in the urine, but in very small amounts of about 1 mg/24 hr, except during pregnancy where it can go up to 70 mg/24 hr. (Vela et al., 1969). Urinary excretion however, was not related to plasma cholesterol levels (Acevedo et al., 1973) and therefore could not be a major route of cholesterol excretion.

4. Milk

Another form of cholesterol secretion limited to the female of mammalian species is through the milk. Human milk cholesterol content has been determined to be 20 mg/dl (Macy et al., 1953), while rat milk cholesterol content has been determined to be between 25-65 mg/dl (Clarenburg et al., 1966; Reiser et al., 1972). The sources of cholesterol in milk may be from the diet, mammary gland synthesis and from endogenous synthesis other than the mammary gland. The cholesterol content of milk in the rat can be increased by cholesterol feeding (Reiser et al., 1972) and an estimated 11% of milk cholesterol comes from dietary cholesterol, which was determined in rats on a 0.05% cholesterol diet (Clarenburg et al., 1966).

5. Hormone Production

The adrenal glands, ovaries and testes convert cholesterol to steroid hormones. As indicated previously, the ovary was found to produce over 75% of its own cholesterol requirement, whereas the adrenal gland only produced about 4% (Turley et al., 1981a;1981b). The additional cholesterol required was supplied by plasma lipoproteins. Studies performed on rats have indicated that when plasma cholesterol decreases, adrenal gland cholesterol synthesis increases and the intracellular stores of cholesteryl ester decrease (Andersen et al., 1976). The process can be reversed by raising the plasma cholesterol level through the infusion of cholesterol carrying lipoproteins (Balasubramaniam et al., 1977). Therefore, the low adrenal gland cholesterol synthesis activity has been postulated to be due to the adrenal

gland receiving its cholesterol requirements from plasma lipoproteins. Rat high density lipoprotein (see section II.A.1. Methodological Classification) appears to be the principle supplier of cholesterol to the adrenal gland (Andersen et al., 1976;1977). Moreover, high density lipoprotein binds with high affinity to the rat testes and appears to be the main exogenous source of cholesterol to that organ (Andersen et al., 1977;1978; Chen et al., 1980) however, a high amount of HMG CoA reductase activity is also present in the rat testes, so most of the cholesterol needed for steroid hormone synthesis is produced endogenously and not supplied by high density lipoprotein. In addition, low density lipoprotein appeared to bind with high affinity to human fetal testes and bovine corpus luteum indicating that high density lipoprotein as a source of cholesterol to gonadal tissue may be species specific (Brown et al., 1979).

Cholesterol homeostasis in human steroid hormone producing cells has been postulated to consist of four stages. The first stage being the basal stage, when the cell produces hormone at a steady state. The cell has a large storage supply of cholesteryl ester and plasma lipoprotein-cholesterol is received by lipoprotein receptors (see section II.C.4. LDL Catabolism). HMG CoA reductase activity is also at a steady state. The second stage occurs when the gland receives stimulation to increase steroid output, as when ACTH stimulates the adrenal gland. Unesterified cholesterol is produced by the hydrolysis of esterified cholesterol present within the cell and HMG CoA reductase is stimulated into producing more unesterified cholesterol for steroid hormone synthe-

sis. The third stage is reached upon prolonged stimulation of hormone production, when the intracellular cholesteryl ester stores are depleted, the bulk of cholesterol for hormone production is obtained from the plasma by increasing the number of receptors on the cell surface for plasma lipoproteins. HMG CoA reductase activity then becomes inhibited by the increased plasma cholesterol input to the cell and endogenous synthesis of cholesterol decreases. The fourth stage occurs at the end of hormone production stimulation as the cell goes back to the basal state. Lipoprotein receptors decrease and cholesteryl esters accumulate within the cell until the cell is back to the first stage condition (Brown et al., 1979). Although hormone production is a major function of cholesterol, it does not appear to be a major source for plasma cholesterol clearance, due to the availability of intracellular stores and intracellular synthesis of cholesterol in steroid hormone producing cells.

II. LIPOPROTEINS

A. Lipoprotein Classification

1. Methodological Classification

Nomenclature of the various classes of lipoproteins is dependent on the method of isolation and characterization of the lipoprotein classes. Alaupovic (1980) proposed two types of lipoprotein nomenclatures (1) operational and (2) chemical. Operational nomenclature is dependent on the method of separation of the various lipoprotein

classes. The nomenclature for electrophoretic separation would include lipoproteins remaining at the origin called origin lipoproteins, $alpha_1$ lipoproteins are those lipoproteins which migrate in the $alpha_1$-globulin fraction and $alpha_2$ lipoproteins are those which migrate in the $alpha_2$-globulin fraction, while beta lipoproteins migrate in the beta-globulin fraction.

Nomenclature based on hydrated density for human lipoproteins would include chylomicrons with densities less than 0.94 g/ml, very low density lipoproteins (VLDL) with density greater than 0.94 g/ml and less than 1.006 g/ml, low density lipoproteins (LDL) have densities between 1.006-1.063 g/ml, high density lipoproteins (HDL) have a density range from 1.063-1.210 g/ml and very high density lipoproteins (VHDL) have a density greater than 1.210 g/ml. Two subfractions of HDL isolated from human plasma are HDL_2 and HDL_3 which have densities of 1.063-1.125 and 1.125-1.210 g/ml respectively (Nicoll et al., 1980b). Ultracentrifugation has also allowed for the identification of other subfractions of the major classes of lipoproteins such as HDL_{2a}, HDL_{2b} (see section II.B.1. Ultracentrifugation), HDL_1 and HDL_c (see section II.D. Rat Lipoprotein) (Lindgren, 1979).

2. Lipoprotein Families

Chemical nomenclature is based on the concept that plasma lipoproteins consist of lipoprotein families or particles which are characterized by distinct apolipoproteins. Lipoprotein families are present as free, separable forms or association complexes. Lipoprotein families are polydisperse and display antigenic homogeneity. Apolipoproteins are

designated by capital letters; constitutive polypeptides by Roman numerals; and the polymorphic forms of apolipoproteins or polypeptides by Arabic numbers. Examples of this nomenclature are as follows: The apolipoprotein A (apo A) family consists of two polypeptides A-I and A-II and the apolipoprotein C (apo C) family has three polypeptides C-I, C-II and C-III, whereas apolipoprotein B (apo B), apolipoprotein D (apo D), apolipoprotein E (apo E) and apolipoprotein F (apo F) are believed to consist of only one polypeptide each (Alaupovic, 1980). Throughout this dissertation lipoproteins will be referred to primarily by operational nomenclature with mention of the constitutive substances as necessary.

B. Methodologies for Lipoprotein Separation

1. Ultracentrifugation

Isolation of soluble lipoprotein of constant composition was first accomplished in 1929 from horse plasma by precipitation using half saturated ammonium sulfate (Macheboeuf, 1929). Twelve years later, the lipoprotein fraction isolated by precipitation was found to migrate in the $alpha_1$-globulin region using paper electrophoresis (see section II.B.2. Electrophoresis), which would indicate the lipoprotein fraction was equivalent to HDL (Macboeuf et al., 1941). Precipitation by ammonium sulfate was not an ideal technique for lipoprotein separation, therefore other techniques for lipoprotein fractionation were devised, one of which was ultracentrifugation.

Since lipoproteins have densities lower than other naturally occuring macromolecules, changes in solvent density has allowed the sep-

aration of plasma lipoproteins from other macromolecules by floatation in a high centrifugal field. The equation for the velocity of sedimentation coefficient of a macromolecule (S) is as follows:

$$S = (1/\omega^2 x)\ (dx/dt) = M(1-V\rho)/3\pi N\eta d.$$

S is in Svedberg units (10^{-13} seconds), ω is the angular velocity in radians/sec = $(2\pi)/60$rpm, x is the distance (cm) of particle from the rotation axis, t is the time in seconds, M is the molecular weight (g), V is the partial specific volume of macromolecules (cm^3/gm), ρ is the density of the solvent (g/cm^3), N is Avogadro's number, η is the viscosity of solvent (poise) and d is the diameter of the particle (cm). The S value for chylomicrons is 10^3-10^5 for VLDL is 12-400 and for LDL is 0-12, while HDL is found in the sediment (Hatch et al., 1968). The interpretation of analytical ultracentrifugal results obtained for plasma was the major problem needed to be overcome in order to use this technique for lipoprotein analysis. This problem was resolved and plasma lipoproteins have been characterized by analytical ultracentrifugation (Gofman et al., 1949). Unfortunately the analytical ultracentrifuge is a very costly and complicated piece of equipment and few laboratories in the world have one. Moreover, the stress forces exerted on the lipoproteins subjected to analytical ultracentrifugation cause reversible and irreversible changes in lipoprotein structure and characteristics (Lindgren, 1980).

The most commonly used method in research for lipoprotein analysis is sequential ultracentrifugation (Chapman, 1980) and the most fre-

quently used sequential ultracentrifugal technique involved adjusting the plasma sample to a higher density by the addition of salt, either in solution or by dry weight. The sample would then be centrifuged at high speed for long periods of time, usually from 18-48 hours. Following centrifugation the fraction containing lower density lipoproteins would be at the top of the centrifuge tube separated from the higher density fraction at the bottom of the tube. The tube could then be sliced between the two lipoprotein fractions or the floating lower density fraction could be removed from the top by pipette (Havel et al., 1955). Ultracentrifugal techniques appear to be the best way currently available to fractionate human HDL into the subfractions of HDL_{2a} HDL_{2b} and HDL_3 (Lindgren et al., 1979). Table 1 demonstrates the results of human and other species lipoproteins isolated by sequential ultracentrifugation and analyzed for protein, phospholipid, triglyceride and both esterified and unesterified cholesterol (Havel et al., 1955).

Variations of ultracentrifugal techniques have been developed all based on the same principle of density adjustment with centrifugal force used in separating the various density lipoprotein fractions. Each technique has advantages over the other, but all suffer from some limitations and differences in lipoprotein constituent values have been obtained using different ultracentrifugal techniques (Hojnacki et al., 1978; Shireman et al., 1983). Ultracentrifugation can lead to lipoprotein degradation (Fainaru et al., 1977) and loss of cholesterol from the various lipoprotein fractions (Marcel et al., 1981). LDL has been demonstrated to be significantly affected by ultracentrifugation, apo A-I

is lost from HDL and salt gradients which tend to set up prior to separation also interfere with lipoprotein resolution (Lindgren et al., 1979). Another drawback to ultracentrifugation is the time involved in separating lipoprotein fractions, which can take up to a week to accomplish (Havel et al., 1955).

2. Electrophoresis

Apolipoproteins on the surface of lipoprotein molecules display a net charge which determines electrophoretic mobility and allows separation of lipoprotein classes by electrophoresis. The lipoprotein net charge is mostly determined by the balance of charges on terminal and side-chain amino acid residues of the apolipoproteins. Additional charge contributions could be from absorbed metal cations and fatty acid anions. Phospholipids contribute little to lipoprotein electrophoretic mobility, since over 90% of the phospholipids present in lipoproteins exist in the zwitterionic state at physiological and electrophoretic pH values (Hatch et al., 1968).

Ultracentrifugal separation of lipoproteins has often been correlated with various other methods for lipoprotein separation. The lipoprotein bands obtained by paper electrophoresis have been correlated to ultracentrifugally isolated lipoproteins. The density equivalents for lipoproteins separated by paper electrophoresis has been determined as follows: $alpha_1$ lipoprotein corresponds to HDL density 1.063-1.210 g/ml $alpha_2$ lipoprotein also known as prebeta lipoprotein corresponds to VLDL density less than 1.006 g/ml, beta lipoprotein corresponds to LDL with density between 1.006 and 1.063 g/ml and the band at the origin corre-

sponds to chylomicrons (Lees et al., 1965; Levy et al., 1966). Paper electrophoresis, because it is less time consuming, easier to run and correlates well with the ultracentrifugal methodology has been used to phenotype human lipoprotein disorders previously only definable by ultracentrifugal techniques (Fredrickson et al., 1965). Unfortunately, paper electrophoresis like ultracentrifugation has some problems. Resolution of lipoprotein bands by paper electrophoresis was poor until it was improved by substituting a barbital-albumin buffer for the barbital buffer used previously (Lees et al., 1963), however it still required 16 hours for an electrophoretic run.

Cellulose acetate used as a support medium for electrophoresis was found to be capable of excellent resolution of plasma lipoproteins without the requirement of albumin in the buffer within 15-45 minutes (Chin et al., 1968; Beckering et al., 1970; Fletcher et al., 1970). The use of agarose gels and polyacrylamide gels for lipoprotein electrophoresis does not markedly improve resolution of the lipoprotein fractions over that of cellulose acetate nor is there any added convenience in the use of gels (Conlon et al., 1979; Muniz, 1977). Cellulose acetate electrophoresis is a suitable and convenient method for lipoprotein fractionation (Hatch et al., 1968).

Currently, isotachophoresis and isoelectric focusing are used in lipoprotein research for the identification and characterization of apolipoproteins (Bon et al., 1981; Eggena et al., 1972). The lipoproteins are usually first isolated by ultracentrifugation, delipidated then electrophoresed (Eggena et al., 1972). Delipidation however, does not

always appear to be necessary (Bon et al., 1981; Bugugnani et al., 1984; Godolphin et al., 1974), yet with nondelipidated lipoprotein fractions isolated by high resolution electrophoretic techniques, the number of subfractions resolved increases tremendously. However, the increased number of subfractions observed using high resolution techniques such as, isoelectric focusing could be due to different distributions of lipid content within the same class of lipoprotein (Rosseneu-Motreff et al., 1969) or protein interaction with ampholytes (Hare et al., 1978). Therefore, the number of subfractions of lipoproteins observed using high resolution techniques could be due to the techniques and not to the actual number of subfractions.

Accurate quantitation of electrophoretically-separated plasma lipoproteins with non-specific "total" lipid stains has been difficult, because of each lipoprotein's differing affinities for such stains (Fletcher et al., 1970). Such commonly used lipid stains as Sudan black B, preferentially stain unsaturated sterol esters and unsaturated triglycerides, whereas unesterified cholesterol and phospholipids are stained negligibly or not at all (Schjeide et al., 1963). Moreover, the various cholesterol esters and triglycerides have different staining affinities for Sudan black B, dependent upon their particular fatty acids. Oil red O, which is another commonly used "total" lipid stain, stains cholesterol esters more intensely than Sudan black B, but its staining is also dependent on the cholesterol ester's degree of fatty acid saturation. In addition, oil red O also stains triglycerides as well as albumin, but does not stain phospholipids or unesterified cho-

lesterol. Recently a cellulose acetate electrophoretic technique, which combines an enzymatic staining procedure for total cholesterol (Allain et al., 1974) has been developed for determining human plasma lipoprotein-cholesterol concentrations which are currently employed as risk factors for human atherosclerotic disease (Cobb et al., 1978). The enzymatic assay used cholesterol oxidase, which is specific for 3β sterols and requires a double bond in the delta 5 or delta 4 positions of the steroid molecule. Interference from sterols normally occurring in human plasma using the enzymatic staining technique is less than 0.1% the total plasma cholesterol concentration indicating the technique has a high specificity for cholesterol in plasma (Richmond, 1973). The method of quantitating human plasma lipoproteins using enzymes specific for cholesterol is more precise and accurate as opposed to "total" lipid staining.

3. Polyanionic Precipitation

Another group of methods used to separate and identify lipoproteins is polyanionic precipitation of which the most commonly used are manganese heparin or dextran sulfate precipitation (Burstein et al., 1960; 1970; 1973). Precipitation techniques are usually rapid and can be adjusted to clear solutions of any particular class of lipoprotein merely by titrating the anion concentrations. Lower density lipoprotein classes are found in the precipitate while higher density lipoproteins remain in the supernate. Therefore, a chylomicron free solution can be achieved by adding a low concentration of a polyanionic precipitating agent and a VLDL/chylomicron free solution can be achieved by adding a

higher concentration of precipitating agent. Precipitation of each lipoprotein fraction can be done sequentially until the solution is lipoprotein free. Recently, precipitation techniques have become more refined and have been used to separate the HDL subfractions HDL_2 and HDL_3 (Gidez et al., 1982).

Although HDL subfraction precipitation correlates well with ultracentrifugal studies, there is incomplete separation of HDL_3 from HDL_2, therefore the technique is only useful if a precise measurement of the two HDL subfractions is unnecessary (Daerr et al., 1983). This is indicative of the major problem with precipitation methods, which is that although the various lipoprotein density classes can be sequentially precipitated, the resolution between lipoprotein classes is so close that a portion of the lipoprotein class which is desired in solution may also occur in the precipitate (Burstein et al., 1970). In a study comparing various precipitation techniques to sequential ultracentrifugation (Warnick et al., 1979a; 1979b), a good correlation was found between the methods however, differing HDL-cholesterol levels were obtained depending on which method was used. This would indicate that the various precipitation techniques are not equivalent and can lead to significant differences in HDL-cholesterol quantitation. Another problem with precipitation techniques is the inadvertent precipitation of HDL containing apolipoprotein E along with the apolipoprotein B-containing lipoproteins (Mahley et al., 1977b).

4. Column Techniques

Many column techniques for obtaining pure lipoprotein fractions require ultracentrifugal separation from the rest of plasma proteins prior to applying the sample to the column. Agarose column chromatography for example which separates lipoproteins by size requires prior purification by ultracentrifugation (Rudel, 1974). Heparin affinity chromatography is a good technique for isolating beta lipoprotein and those lipoprotein fractions containing apo E, especially HDL containing apo E, however purification of the isolated fractions requires further manipulation, which can affect the nature of the lipoprotein being studied (Bentzen et al., 1982). A new rapid lipoprotein separation technique coupling high performance liquid chromatography (HPLC) with exclusion chromatography can use plasma volumes of two microliters, but sacrifices resolution, due to loss of sensitivity from using small plasma volumes. Therefore, only two plasma lipoprotein fractions, HDL and LDL, could clearly be isolated from human plasma. (Ohno et al., 1981). Improvement in the HPLC technique will continue to occur and with the use of larger plasma volumes the resolution between lipoproteins may be enhanced, which would make HPLC an excellent rapid technique for isolating plasma lipoproteins in pure form.

5. Immunoassay of Apolipoproteins

Immunological techniques were developed to quantitate the apolipoprotein concentration within plasma as an easier alternative to ultracentrifugal isolation. The first methods for apo A-I and apo B, the

major apolipoproteins of HDL and LDL respectively, involved a double antibody precipitation procedure which required plasma lipoprotein delipidation and 60 hours of incubation (Schonfeld et al., 1974a; 1974b). A radioimmunodiffusion assay was later developed for Apo A-I that correlated well with the double antibody procedure and with the HDL-cholesterol concentration in plasma (Albers et al., 1976). Moreover, this method was easier to perform, although, delipidation was still necessary and the incubation time required 48-72 hours. Recently, an improved radioimmunoassay system for apo A-I was developed which used a detergent that removed the necessity for plasma lipoprotein delipidation. In addition, an immunoglobulin absorbent was used which removed the free from the bound antibody, eliminating the necessity for a second antibody. This system required only a 10 minute incubation and correlated well with double antibody techniques (Maciejko et al., 1982). Moreover, the new apo A-I radioimmunoassay technique also correlated well with HDL-cholesterol concentrations (Maciejko et al., 1983).

Apo B quantitation using immunological techniques appears to be best achieved by electroimmunoassay as demonstrated by its comparison to radioimmunoassay and radioimmunodiffusion, since the latter two methods appear to underestimate the amount of apo B present. Moreover, the electroimmunoassay appears to be the easiest to perform (Curry et al., 1978). A more recent assay has been developed using a competitive enzyme linked immunoassay for apo B substituting changes in enzyme activity for radioactivity. This technique has correlated well with angiographically described coronary artery disease (Vander Heiden et

al., 1984).

Any immunoassay is dependent on the purity of the antigen for which the antibody is produced. Apo A-I is a relatively easy apolipoprotein to isolate and purify, while Apo B appears to be extremely difficult to isolate and purify (Bradley et al., 1980). Another difficulty with assays for apolipoproteins are that they may be found within different classes of lipoproteins. Apo A-I is found in chylomicrons, VLDL and HDL, although mostly in HDL, whereas apo B is found in VLDL and chylomicrons as well as LDL (Smith et al., 1978). Epidemiological work is currently underway to ascertain the significance of apolipoprotein measurement with regard to predicting atherosclerotic disease and the extent of coronary artery disease with some promising preliminary results (see section V.C. Lipoproteins and Atherosclerosis). However, apolipoprotein immunoassays greatest benefits may be in the explanation of the causes for atherosclerosis and the further understanding of lipoprotein metabolism (Blackburn, 1983).

C. Lipoprotein Synthesis and Metabolism

1. Triglyceride Rich Lipoprotein Synthesis

Triglyceride rich lipoproteins include both chylomicrons and VLDL which are produced in the intestine and liver respectively. The liver in the fasting state produces about 80% of the plasma's triglyceride rich lipoproteins (Windmueller et al., 1968). The core lipids which include triglycerides and cholesterol esters are synthesized on the

cytoplasmic surface of the endoplasmic reticulum (ER) (Coleman et al., 1978; Hashimoto et al., 1980; Lichenstein et al., 1980). ACAT esterifies cholesterol in the rough ER and then esters are transferred to the smooth ER (Hashimoto, 1980). Enzymes responsible for cholesterol synthesis are also in the ER and the specific activity of HMG CoA reductase is highest in the smooth ER (Havel et al., 1973b), so the lipid core and surface lipids of the lipoprotein can all be assembled in the same area of the cell.

The apolipoproteins are probably synthesized on ribosomes bound to the rough ER (Alexander et al., 1976). Exactly where the lipoprotein particles are packed is still unclear although there is some evidence it occurs on the smooth ER (Alexander et al., 1976). It has been proposed that during synthesis the apolipoprotein is inserted into the lipid bilayer of the ER. Apolipoprotein is being synthesized at the same time the core and surface lipids of the lipoprotein are being synthesized. The nascent lipoprotein buds off from the ER with the apolipoprotein attached when an excess amount of lipids are accumulated. Lipid droplets may bud off without the apolipoprotein and accumulate in the cell when high cholesterol concentrations are present intracellularly, which may be reflected by high plasma cholesterol concentrations (Swany, 1980).

The lipoproteins after packaging are transferred into the cisternae and secretory vesicles of the Golgi complex. It was postulated that specialized tubules transfer the nascent lipoproteins to the Golgi apparatus from the ER (Alexander et al., 1976), although it is also pos-

sible that the lipoproteins are transported via vesicular transport (Novikoff et al., 1978). Lipoproteins isolated from the Golgi apparatus are similar to VLDL found in the plasma except Golgi lipoproteins contain more phospholipids and fewer apolipoproteins (Hamilton, 1980). The nascent lipoprotein particles are packaged in secretory vesicles and transported to the sinusoidal surface of the cell where the vesicles fuse with the plasma membrane and the lipoproteins are released. VLDL is released into Disse's space of the liver and chylomicrons are released into the basolateral spaces between intestinal absorptive cells (Hamilton et al., 1967; Hamilton, 1972).

2. Triglyceride Rich Lipoprotein Catabolism

Extrahepatic lipoprotein lipase EC 3.1.1.34 (LPL) (Nomenclature Committee, 1979) is an enzyme bound to the endothelial luminal surface of capillaries which supply the tissues that utilize circulating triglycerides (Scow et al., 1972). LPL forms free fatty acids and glycerol from triglycerides and the rate of the reaction is strongly dependent upon the particular lipoprotein present (Fielding, 1970). A protein cofactor present in VLDL and in chylomicrons was found to be responsible for increased LPL activity (Havel et al., 1973a). Further investigation revealed that the protein cofactor responsible for lipoprotein lipase activity was apo C-II (LaRosa et al., 1970). Moreover, the amount of apo C present in VLDL was found to be dependent on the triglyceride concentration of VLDL (Eisenberg et al., 1975). Chylomicrons have been demonstrated to receive apo C from HDL (Havel et al., 1973c). In addi-

tion, apo C was shown to transfer from HDL to VLDL as the triglyceride concentration of plasma increases, while as lipolysis occurred apo C was transferred back to HDL (Kashyap et al., 1977).

Two forms of lipoprotein lipase are released into the plasma of rats and humans upon in vivo administration of heparin (Krauss et al., 1973; 1974). One form of lipoprotein lipase was inhibited by protamine, while the other form was resistant to protamine. The protamine resistant lipoprotein lipase was found to be hepatic in origin, whereas the protamine sensitive lipoprotein lipase was found to originate from the capillary endothelium. The activity of the hepatic lipoprotein lipase appeared to be less than that of the extrahepatic lipoprotein lipase, since VLDL was found to be a poor substrate for hepatic lipoprotein lipase and an excellent substrate for extrahepatic lipoprotein lipase. Hepatic lipoprotein lipase has been suggested to have a greater specificity for lipoprotein remnants, which are those lipoproteins that have already been acted upon by extrahepatic lipoprotein lipase (Krauss et al., 1973; 1974). Moreover, hepatic lipoprotein lipase has been found to have phospholipase activity (Enholm et al., 1975) and appears to be localized exclusively on the surface of hepatic endothelial cells (Kuusi et al., 1979). Hepatic lipoprotein lipase appears to act on smaller lipoprotein molecules preferring VLDL to chylomicrons and the lower density IDL to VLDL (Nicoll et al., 1980a).

Removal of human plasma chylomicrons from the circulation is extremely rapid, since chylomicrons have a half life of about 5 minutes (Grundy et al., 1976; Grundy, 1978). Cholesterol incorporated into chy-

lomicrons injected into rats was rapidly taken up by the liver with at least 93% of the injected cholesterol found in the liver within 1 hour after injection (Goodman, 1962). Another study also using rats found after 10 minutes only 20% of the chylomicron triglycerides in the liver although, 80% of the injected cholesterol incorporated into chylomicrons was found in the liver, (Redgrave, 1970). These results were duplicated in sheep and dogs (Bergman et al., 1971), indicating that extrahepatic lipoprotein lipase removed triglyceride prior to uptake by the liver of the chylomicron shell which contained the remaining esterified cholesterol core. Humans with extrahepatic lipoprotein lipase deficiency have very high levels of triglycerides within chylomicrons, which are not taken up the liver. This would be consistent with the proposed specificity of hepatic lipoprotein lipase (Nicoll et al., 1980) and suggests that a change must be made in the chylomicron molecule by extrahepatic lipase as triglyceride is removed and a chylomicron remnant is formed. The change in the chylomicron molecule may be the loss of apo C as triglyceride lipolysis occurs, since apo C may inhibit chylomicron binding to the hepatic lipoprotein receptor (Shelburne et al., 1980; Windler et al., 1980a). Receptor mediated rapid uptake of lipoproteins containing apolipoproteins B and or E by the liver (Brown et al., 1981) will be discussed further (see section II.C.4. LDL Catabolism).

3. VLDL Catabolism and LDL Formation

Triglyceride rich VLDL formed and released from the liver, circulates in the plasma and is acted upon by extrahepatic and hepatic lipoprotein lipase which decreases the triglyceride content of VLDL, causing an increase in VLDL density. VLDL forms intermediate density lipoprotein (IDL), which has a hydrated density between 1.006-1.019 g/ml. The continued action of lipoprotein lipase on IDL results in the production of LDL with a density between 1.019-1.063 g/ml. This precursor product relationship of VLDL and LDL was found by the injection of labeled VLDL into humans and rats (Eisenberg et al., 1973a; 1973b). Apolipoprotein B was found to be produced in the liver and intestine of the rat (Schonfeld et al., 1978) and is a component of chylomicrons, VLDL and LDL (Schaeffer et al., 1978b). The apolipoprotein composition of LDL is almost completely apo B (Schaefer et al., 1978b), therefore as VLDL loses triglyceride by the action of lipoprotein lipase the apo B component of VLDL remains in the plasma, but each LDL particle becomes more concentrated with apo B (Eisenberg et al., 1973a; 1973b). Moreover, in vitro incubation of VLDL with lipoprotein lipase rich plasma produced IDL particles (Eisenberg et al., 1975). Also, the conversion of VLDL to LDL was found to be a one way process, since injection of labeled LDL failed to show any labeled VLDL in human plasma (Langer et al., 1978).

The interaction of VLDL with lipoprotein lipase in humans was found to be less efficient than lipoprotein lipase interaction with chylomicrons (Grundy et al., 1976; Grundy, 1978), since the half life for plasma VLDL was determined to be 1-3 hours, which is much longer than

that for chylomicrons (Bilheimer et al., 1972; Sigurdsson, et al., 1975). However, apo B from rat plasma VLDL was cleared more rapidly from rat plasma than human plasma VLDL from human plasma, but the same precursor product relationship exists between VLDL and LDL in both rat and human (Faergeman et al., 1975). The bulk of rat VLDL appears to be removed directly from the circulation by specific hepatic receptors before VLDL can be fully catabolized to LDL (see section II.C.4. LDL Catabolism), which would partially explain why the LDL concentration of rat plasma is so low (Stein et al., 1974).

4. LDL Catabolism

The fractional catabolic rate of LDL in humans is dependent on LDL clearance rather than LDL formation, since it was found that increases in LDL appear to be due to problems in catabolism rather than increases in LDL production (Sigurdsson et al., 1976). In vitro analysis has shown that fibroblasts when incubated with plasma lipoproteins derive all their cholesterol from LDL (Brown et al., 1974a). Moreover, a specific cell surface receptor that binds LDL with high affinity and specificity was found in cultured fibroblasts (Brown et al., 1974b; Goldstein et al., 1974a; 1976). The specificity of the lipoprotein receptor found in cultured fibroblasts has been determined to be for the apolipoproteins E and B, which are major components of LDL, VLDL and chylomicrons (Innerarity et al., 1978; Mahley et al., 1977c). Electron microscopic analysis demonstrated that LDL bound to the lipoprotein receptor migrated to a coated pit on the cell surface (Anderson et al., 1976; 1977a; 1977b; Orci et al., 1978) which contained 50-80% of the LDL

receptors and appears to be specialized for rapid endocytosis. The receptor bound lipoprotein was ingested through absorptive endocytosis following which, the internalized LDL was incorporated into endocytotic vesicles that fused with lysosomes, where the protein component of LDL was degraded into amino acids and released into the cell culture medium (Goldstein et al., 1974a; 1975). The cholesteryl esters of LDL were hydrolyzed by a lysosomal acid lipase (Brown et al., 1975b) and the unesterified cholesterol was transferred to the cellular compartment where it was found associated with cell membranes (Brown et al., 1975c). Unesterified cholesterol originating from LDL also suppressed HMG Co A reductase (Brown et al., 1973) and activated ACAT facilitating reesterification of cholesterol within the cell (Brown et al., 1975a; Goldstein et al., 1974b). In addition, the LDL receptor was found to be regulated by feedback inhibition, so that when fibroblasts were incubated with LDL, the LDL receptor concentration per cell decreased (Brown et al., 1975d). The fibroblasts in the normal state preferred cholesterol from LDL, since HMG Co A reductase was normally suppressed (Brown et al., 1975c). Therefore, based primarily on in vitro studies of cultured fibroblasts it has been suggested that the primary function of plasma LDL is the transport of cholesterol to peripheral tissues to fulfill the tissue's cholesterol requirements (Brown et al., 1981). Moreover, using membrane binding studies on bovine tissue high affinity LDL receptors similar to those found in cultured fibroblasts were found on the membranes of the adrenal gland, ovary, liver, kidney, myocardium, spleen, brain, erythrocytes, pancreas, thymus, skeletal muscle, testes, lung,

jejunum, ileum and adipose tissue (Kovanen et al., 1979a), evidence that this LDL receptor, also referred to as the classical LDL receptor is found in almost every tissue of the body.

An alternative pathway for LDL degradation has been found to be the scavenger pathway, which has low affinity binding, nonspecific uptake and is dependent on plasma lipoprotein concentration (Goldstein et al., 1974a). The scavenger pathway appeared to be involved in non-specific pinocytosis and is believed to degrade about 15% of human plasma LDL daily (Goldstein et al., 1977b). However, the scavenger pathway did not appear to remove normal LDL from the plasma, but was able to remove LDL which was exogenously modified to be more electronegative by acetylation or maleylation of isolated LDL using organic chemical means. In other words, the scavenger pathway was found to remove LDL which had lysine residues experimentally modified by acetylation or maleylation, thereby increasing LDL's electronegativity (Brown et al., 1980). Acetylation of LDL probably does not occur in vivo (Goldstein et al., 1979) however, LDL isolated from human lymph has been found to be more electronegative than plasma LDL (Reichl et al., 1973; 1975). Moreover, LDL incubated with cultured endothelial cells were found to be more electronegative and had enhanced uptake by incubated macrophages (Henriksen et al., 1981). In addition, in vivo administration of the exogenously modified LDL particles resulted in an increased uptake into rat liver endothelial cells as compared to nonmodified native LDL molecules which were taken up primarily by liver parenchymal cells (Nagelkerke et al., 1984). The nature of any in vivo modification of

LDL has yet to be determined. Another macrophage receptor has been found distinct from the acetylated LDL receptor, which binds LDL complexed with dextran sulfate. However, since dextran sulfate will bind to the receptor in the absence of LDL, the receptor site appears to be selective for dextran sulfate rather than LDL (Basu et al., 1979).

Initially, the scavenger cell pathway was found exclusively in cultured macrophages whereas the classical LDL receptor pathway was found exclusively in parenchymal and connective tissue (Brown et al., 1980). Monocytes incubated in vitro exhibited the characteristics of the classical LDL receptor pathway, but as the monocytes matured they expressed scavenger pathway activity indicating both receptors may exist on the same cell type (Fogelman et al., 1981). Since macrophages are derived from monocytes (Wheater et al., 1979) it is possible that as monocytes mature and leave the circulation they differentiate into macrophages and lose the ability to bind LDL by the classical receptor mechanism, although using immunocytochemical procedures cultured macrophages were observed to express both the classical and scavenger receptor pathways (Traber et al., 1983). Recently, bovine endothelial cells were demonstrated to possess both classical LDL and scavenger receptor pathways (Baker et al., 1984; Stein et al., 1980). However, human endothelial cells may lack LDL scavenger receptors, since endothelial cell do not appear to have lipid deposits, when examined in humans with classical LDL receptor deficiencies (Buja et al., 1979). Lipid laden cells known as foam cells (see section V.C.2. LDL and Cholesterol) found in humans suffering from classical LDL receptor deficiencies were found

to be derived from smooth muscle cells or macrophages (Cookson, 1971) with at least 80% derived from macrophages (Vedeler et al., 1984). These foam cells were found to be distributed in tissues throughout the body, including the stromal cells of the thymus, spleen, skin, kidney and also in the aortic and mitral valves (Buja et al., 1979). The chief source of lipoprotein receptors in the body for both the classical receptor and the scavenger receptors appears to be the liver (Kovanen et al., 1979a), with about 75% of the body's classical LDL receptor present in the liver (Bilheimer et al., 1984; Spady et al., 1983). The liver parenchymal cells which are the hepatocytes (Leeson et al., 1981) contain the classical LDL receptors, whereas the Kupfer cells contain the scavenger LDL receptors (Brown et al., 1980). As previously mentioned, rat liver endothelial cells also appear to have the scavenger cell receptors (Nagelkerke et al., 1984).

The classical LDL lipoprotein receptor pathway has a limit to the amount of cholesterol it will take up due to feedback control on LDL receptors, whereas the scavenger pathway has no limit and appears to be the chief pathway for LDL degradation in humans with an effective lipoprotein receptor deficiency (Goldstein et al., 1977a; 1977b). Moreover, massive accumulation of cholesteryl ester has been found to occur in mouse macrophages incubated in vitro and might account for the widespread deposition of LDL-derived cholesteryl esters found in the macrophages of humans suffering from an effective lipoprotein receptor deficiency (Goldstein et al., 1979). Humans suffering from an effective lipoprotein receptor deficiency have what is known as familial hypercho-

lesterolemia, also known as hyperlipoproteinemia type IIa, which may be due to a lack of classical LDL lipoprotein receptors, inability of LDL to bind to classical lipoprotein receptors or to an inability of the classical lipoprotein receptor LDL complex to be internalized into the cell (Anderson et al., 1977b; Brown et al., 1974b; Goldstein et al., 1976). This condition results in extremely high plasma cholesterol levels and humans suffering from this disorder have a greater potential for developing coronary artery disease (see section V.C.2. LDL and Cholesterol).

5. HDL Synthesis

HDL synthesis and secretion have been demonstrated in the small intestine and in the liver of humans and rats (Green et al., 1978; 1979; Hamilton et al., 1976; Marsh, 1974; 1976; Noel et al., 1974; Roheim et al., 1966). The contributions of the intestine and the liver to HDL has been difficult to determine, even though there is a difference in the apolipoprotein pattern of nascent HDL from each source. HDL from the intestine contains newly synthesized apo A-I and small amounts of apo E and apo C, while HDL from the liver has a high apo E content, but little apo A-I and apo C (Felker et al., 1977; Hamilton, 1976; Imaizumi et al., 1978a; 1978b; Marsh, 1976; Wu et al., 1979). HDL secreted from perfused liver is disc shaped composed of proteins, phospholipids and cholesterol with almost no cholesteryl ester (Hamilton, 1976). The discoidal HDL particles may be formed from triglyceride rich lipoproteins by the actions of lipoprotein lipase following their secretion from the liver and intestine, since VLDL incubated in vitro with lipoprotein lipase

yields particles similar to HDL (Patsch et al., 1978). Moreover, triglyceride rich lipoproteins have been proposed to be a plasma source of HDL due to extrahepatic lipoprotein lipase, which decreases triglycerides from the core of VLDL or chylomicrons shrinking the lipoprotein molecule. The surface of the triglyceride rich lipoproteins, containing apolipoproteins and phospholipids would then bud off, due to the decreased size of the core which it surrounds forming nascent high density lipoproteins (Tall et al., 1978). In addition, this would explain the inverse relationship found between HDL concentration and VLDL concentration (Nikkila et al., 1978; Tall et al., 1978). Further plasma metabolism of HDL involves the enzyme lecithin:cholesterol acyltransferase (LCAT).

6. LCAT

Lecithin:cholesterol acyltransferase, EC 2.3.1.43 (Nomenclature Committee, 1979) abbreviated as LCAT is a plasma enzyme responsible for the conversion of unesterified cholesterol to esterified cholesterol (Glomset, 1962). Lecithin and unesterified cholesterol in the presence of LCAT produce esterified cholesterol and lysolecithin, the fatty acid from lecithin forming an ester linkage with the 3 β hydroxyl group of cholesterol. The source of the fatty acid of the cholesteryl ester is from the C-2 position of lecithin.

LCAT has been shown to be sensitive to sulfhydryl group blockers like sodium p-hydroxymercuribenzoate (PHMB) or p-chloromercuribenzoate (PCMB) (Hellerman et al., 1943), which act as reversible inhibitors of cholesterol esterification (Glomset, 1962). The mercurial sulfhydryl

group blocker inhibit the enzyme by forming a mercaptide with the cystine sulfhydryl group. The process can be reversed with a thiol donating substance such as mercaptoethanol. The reaction using PCMB as the thiol blocker on a protein (P) and the reverse reaction using a thiol donating agent (RSH) would proceed as follows:

$$\text{a. } P\text{-}SH + Cl\text{-}Hg\text{-}C_6H_4COONa \rightarrow P\text{-}S\text{-}HgC_6H_4COONa + HCl$$

$$\text{b. } P\text{-}S\text{-}HgC_6H_4COONa + RSH \rightarrow P\text{-}S\text{-}H + R\text{-}S\text{-}HgC_6H_4COONa$$

(Benesch et al., 1962; Hamilton, 1960). p-Chloromercuriphenylsulfonic acid or p-chloromercuriphenylsulfonate (PCMPS) as shown in figure 2, another mercurial thiol blocking agent (Velick, 1953) used to inhibit LCAT activity (Glomset et al., 1970) is an analogue of PCMB with a sulfate group that enhances the molecule's aqueous solubility (Vansteveninck et al., 1965). An LCAT inhibitor 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (Stokke et al., 1971), which binds to thiol groups (Ellman, 1959), has been found to bind to two of four half cysteine residues found to be associated with the LCAT molecule, indicating that the SH group of at least one of two cystines may be at the active site and that a disulfide cross linked cysteine is probably present within the LCAT molecule (Chong et al., 1983). Large quantities of lysolecithin have also been found to inhibit LCAT activity (Fielding et al., 1972a), but with the addition of albumin LCAT activity returns to normal probably due to loss of lysolecithin from albumin binding (Aron et al., 1978).

At least two protein cofactors present in the plasma appear to

enhance LCAT activity and they have been identified as apo A-I and a subfraction of the apo C family. Moreover, both protein cofactors have been found to be associated with lipoproteins, particularly HDL (Aron et al., 1978; Fielding et al., 1972b; Kostner, 1978). LCAT has been shown to act preferentially with HDL (Glomset, 1970), while LDL and VLDL seemed not to have an effect on LCAT activity, although recently LCAT has been demonstrated to have some activity with LDL (Barter, 1983). The HDL particle secreted from the perfused liver has been found to be a good substrate for LCAT (Hamilton, 1976) and these nascent HDL particles also appear to be similar to those found in the plasma of patients with LCAT deficiency (Forte et al., 1971). In addition, HDL_3, the denser of the HDL subfractions which can be isolated by ultracentrifugal techniques appeared to be the preferred LCAT substrate in humans (Fielding et al., 1971), whereas HDL_2 appeared to inhibit the LCAT reaction (Fielding et al., 1971; Fielding et al., 1972a; Pinon et al., 1980), although LCAT does not appear to be affected by the ratios of apo A-I to apo A-II in HDL subfractions. Moreover, studies have indicated that LCAT may form a complex with HDL_3 and VHDL (Fielding et al., 1980; Jahani et al., 1981). LCAT incubated in vitro with apo A-I and cholesterol liposomes caused LCAT to be inhibited by the accumulation of cholesteryl esters (Chajek et al., 1978), which could explain why LCAT prefers cholesteryl ester poor HDL_3 and is inhibited by cholesteryl ester rich HDL_2. The Vmax of LCAT activity was found to be 2.4-4 times greater for HDL_3 than HDL_2, after incubating plasma with different mixtures of HDL_2 and HDL_3 and the Km for HDL_3 was 43-60 nmol/ml and 167-391 nmol/ml for HDL_2. A pre-

dictive model formulated on the basis of the kinetic studies showed that at physiological concentrations HDL_2 could act as a competitive inhibitor of the cholesterol esterification reaction displacing the effective substrate HDL_3 by the less effective substrate HDL_2 (Barter et al., 1984).

In vivo esterification of cholesterol by LCAT in man has been estimated to be about 0.12 micromoles per ml plasma per hour (Glomset, 1962; Nestel et al., 1967). An in vitro comparison of rat LCAT activity to human showed the initial rate of cholesterol esterification in the rat was approximately four times that of the human (Frolich et al., 1975). However, it is not clear whether this difference is related to substrate concentration, enzyme or enzyme cofactors, since the comparison was not performed on the same substrate. Another difference between human and rat cholesterol metabolism was found to be that in the human the enzyme most responsible for plasma esterification of cholesterol is LCAT whereas in the rat both plasma LCAT and the liver intracellular enzyme ACAT are responsible for the cholesteryl esters found in plasma lipoproteins (Glomset, 1970). This will be discussed more thoroughly later (see section III.B.1. Cholesteryl Ester Transfer and Exchange).

7. HDL Metabolism

The half life of HDL in the plasma is on the order of five to six days (Brown et al., 1981). Most cells utilize the cholesterol from LDL via the LDL receptor pathway for cholesterol needs, however in rat adrenal and gonadal tissue, HDL may be the source of exogenous cholesterol and may regulate sterol synthesis in those tissues (Andersen et al.,

1979). Rat adrenal cells incubated with HDL and LDL took up cholesterol two to three times more from HDL than LDL. Moreover, HDL-cholesterol uptake was enhanced when ACTH was given to the rats before adrenalectomy, but LDL-cholesterol uptake was unaffected (Gwynne et al., 1976). It has been suggested that the uptake of HDL-cholesterol is a receptor mediated saturable process different from the LDL receptor pathway, since only the cholesterol carried by the HDL particle and not the HDL particle itself has been shown to be taken up by the cell (Gwynne et al., 1980). In addition, rat adrenal steroids have been demonstrated to be derived from both HDL and LDL, but in the human only from LDL has adrenal cholesterol uptake been demonstrated (Brown et al., 1979). Cultured cells from rat ovaries obtained cholesterol exclusively from HDL for the production of progesterone whereas, estrogen synthesis was unaffected by lipoproteins present (Weinstein et al., 1980). High affinity binding of HDL has been demonstrated in the rat testes, for which VLDL competed but LDL did not. Moreover, the binding of HDL to the gonadal tissue showed a two fold increase, when human chorionic gonadotropin was added without an increase in affinity (Chen et al., 1980). Evidence has been given for the existence of a lipase in the adrenals of rats and humans similar in nature to hepatic lipoprotein lipase (Jansen et al., 1981). It has been proposed that hepatic lipase favors the hydrolysis and removal of phospholipids from HDL. This increases the relative amount of unesterified cholesterol on the surface area of the HDL particle, which would facilitate the influx of unesterified cholesterol into those cells which had hepatic lipoprotein lipase like activity. Since

lipoprotein lipase activity similar to hepatic lipase has been found on the adrenals of humans and rats as well as the ovaries of rats, this could explain the specific uptake of HDL-cholesterol in those tissues (Jansen et al., 1980).

Specific lipoprotein receptors as mentioned previously (see section II.C.4. LDL Catabolism) have an affinity for HDL containing apo E, although there may be a qualitative and or quantitative difference between the rat and human LDL receptor (Drevon et al., 1981). The lipoprotein receptor for LDL, which has been found on rat fibroblasts and smooth muscle cells has an affinity for in decreasing order: chylomicron remnants, VLDL, LDL and HDL (apo E containing lipoproteins). Moreover, these affinities are increased if the lipoproteins are cholesterol enriched (Innerarity et al., 1980). Due to the long half life of HDL without apo E it is unlikely that a specific lipoprotein receptor exists for HDL as it does for LDL. LDL, VLDL and HDL_c were found to affect cultured swine aortic smooth muscle cell HMG Co A reductase activity in a concentration related response, yet HDL could not lower the enzyme's activity more than 25% (Assman et al., 1975). In addition, no high affinity binding of HDL has been detected, although low affinity uptake has been noted in fibroblasts probably due to nonspecific pinocytosis (Miller et al., 1977). Also, the binding of HDL was unaffected by incubation with fibroblasts from patients with familial hypercholesterolemia (Miller et al., 1978), further proof that HDL does not compete with LDL for binding on the classical lipoprotein receptor.

HDL has been postulated to remove cholesterol from the surface of

cells assisted by the actions of LCAT. LCAT could continuously create space on the surface of the HDL molecule for unesterified cholesterol to accumulate as the esterified cholesterol was internalized into the core of the molecule and in this way HDL would attract more unesterified cholesterol from cell surfaces (Glomset et al., 1973). It has been reported that the uptake of cellular unesterified cholesterol by HDL is determined by the rate of desorption of cholesterol out of the cell membrane into the aqueous phase surrounding the cell as well as the distance of diffusion to HDL (Phillips et al., 1980). The rate of cholesterol efflux from cells may also be modulated by apolipoprotein A-I (Fielding et al., 1981b). Moreover, macrophage hydrolysis of cholesteryl esters and excretion into the medium was found to be dependent on cholesterol acceptors present in the incubation medium, particularly HDL (Ho et al., 1980). Therefore HDL probably acts as a cholesterol scavenger lipoprotein, accumulating cholesterol from the surface of cells and apparently does not compete with LDL for receptor binding either through the classical lipoprotein receptor pathway or the scavenger lipoprotein receptor pathway.

D. Rat Lipoprotein

1. Composition Compared to Human

No other species outside of humans has been studied more extensively as to their lipoprotein content and metabolism as the rat. Many remarkable similarities can be found between rat and human lipoproteins,

especially in the structure of the apolipoproteins and also some disparities can be found such as the lipoprotein and apolipoprotein distribution (Chapman, 1980). Comparison of HDL apolipoproteins between rat and human using SDS polyacrylamide gel electrophoresis appeared to indicate that the major HDL apolipoprotein, A-I, in both species was identical in terms of electrophoretic mobility. Apo A-II however, was found to be a minor component of rat serum HDL and a major component of human serum HDL (Swaney et al., 1974). Apo A-IV and arginine-rich apolipoproteins were first found in rat HDL and not human HDL, however apo A-IV has since been found in human VLDL (Beisegel et al., 1979). Apo A-II appears to be in monomeric form in the rat as well as in other species including the rabbit and monkey, whereas it is in dimeric form in the human. The minor apo C proteins found in HDL and VLDL are homologous to those of the human (Herbert et al., 1974). Arginine rich lipoprotein also known as apo E (Smith et al., 1978) has been found in the human as a minor component of HDL and a major component of VLDL and chylomicrons (Havel et al., 1973c). In contrast, rat apo E appears to be a major constituent of the HDL subfraction HDL_1 and VLDL (Quarfordt et al., 1978; Weisgraber et al., 1977).

Data on rat lipoprotein composition is very inconsistent and varies with strain, diet, and methods used for isolation (Chapman, 1980). Table 2 indicates the composition of rat lipoprotein as compared to human lipoprotein using similar isolation techniques. Ultracentrifugal separation of rat plasma demonstrated that LDL had a density range between 1.040-1.050 g/ml, and an HDL/LDL overlap which occurred between

densities 1.050-1.063 g/ml (Lasser et al., 1973), in contrast to human HDL which has a more distinct separation between HDL and LDL (Chapman, 1980). No lipoprotein was found in the density fraction between 1.006-1.030 g/ml of normal rats however, rats fed a high cholesterol diet had lipoproteins which appeared in the 1.006-1.030 g/ml fraction, whereas lipoproteins decreased in the density 1.070-1.210 g/ml fraction (Lasser et al., 1973). Ultracentrifugal separation of rat plasma VLDL (density less than 1.006 g/ml), LDL (density 1.006-1.040 g/ml) and HDL (density 1.063-1.210 g/ml) followed by agarose gel electrophoresis indicated that the lipoproteins had homogenous bands with HDL migrating in the prealbumin region. It was also found upon ultracentrifugation of rat plasma that in the density range between 1.040-1.063 g/ml LDL and HDL cross contaminate (Koga et al., 1969). Besides the differences in the major lipoprotein fractions between rat and human, a difference is apparent between rat and human HDL subfractions. Initially, rat HDL was subfractionated into density components corresponding to human HDL_2 and HDL_3 by preparative ultracentrifugation. Both HDL_2 and HDL_3 were characterized by negative staining and electron microscopy. Rat HDL_2 isolated between the densities 1.063-1.125 g/ml appeared to be homogeneous spherical particles, whereas rat plasma HDL_3 appeared to be heterogeneous in size and shape (Pasquali-Ronchetti et al., 1975). In contrast, using rate zonal ultracentrifugation for isolating rat plasma HDL subfractions only HDL_1 and HDL_2 subfractions could be identified and no subfraction corresponding to human HDL_3 appeared (Oschry et al., 1982). Currently, the evidence indicates that the rat has only two HDL subfrac-

tions as compared to three for the human.

2. Rat Plasma Lipoprotein Electrophoretic Patterns

Paper electrophoresis of rat plasma using a Sudan black B stain revealed alpha, beta and prealbumin lipoprotein bands among the 5 major plasma protein bands. An $alpha_2$ band which appeared in rat serum after a high cholesterol diet was found to be unstable, due to changes which occurred in the band upon storage of the hypercholesterolemic samples (Watson, 1961). Agarose gel electrophoresis of rat whole plasma combined with a Sudan black B stain also appeared to suggest a prealbumin lipoprotein band, however in this system lipid-stained bands migrated consistently farther than their corresponding amido black protein-stained bands (Johansson et al., 1976). Human plasma $alpha_1$ lipoprotein also separated by agarose gel electrophoresis appeared to have the same electrophoretic mobility as albumin, with the lack of electrophoretic resolution between albumin and $alpha_1$ lipoprotein possibly being due to nonspecific albumin staining (Johansson et al., 1976; Schjeide et al., 1963). In addition, agarose starch gel electrophoresis of rat plasma using a combined oil red O and fat red 7B "total" lipid stain also appeared to suggest bands which had prealbumin, $alpha_1$, prebeta and beta electrophoretic mobility (Chalvardjian, 1971). The prealbumin and $alpha_1$ bands appeared to correspond to HDL, since after ultracentrifugal fractionation of the plasma, these two bands had a density range greater than 1.063 g/ml, while the prebeta band corresponded to VLDL, and the beta band corresponded to LDL. However, no attempt was made to deter-

mine the actual cholesterol content of the prealbumin-migrating lipoprotein band visualized by a "total" lipid stain.

3. Metabolism and Response to Hypercholesterolemia

Lipoprotein composition may change with dietary status. However, the cholesterol to protein ratio for HDL did not change in rats fed high cholesterol diets, indicating that the amount of cholesterol found in the rat plasma HDL fraction reflects the number of HDL particles present. In contrast, the rat plasma LDL cholesterol to protein ratio increased in rats fed a high cholesterol diet suggesting more cholesterol was being carried by less protein, therefore plasma LDL-cholesterol concentration appears not to be as good an indicator of the total number of plasma LDL particles in the cholesterol fed rat as the plasma HDL-cholesterol concentration is. Yet without changing the amount of cholesterol in the diet, the cholesterol to protein ratios for all rat plasma lipoproteins remained constant, therefore plasma lipoprotein-cholesterol concentrations may directly relate to plasma lipoprotein concentrations (Frnka et al., 1974). Dietary induced hypercholesterolemia produced lipoprotein profile changes in the rat including the appearance of two different lipoprotein fractions, a VLDL with beta electrophoretic mobility and an HDL of lower density referred to as HDL_c (Mahley et al., 1977a). HDL_c has been found to have an $alpha_2$ mobility using paper and agarose gel electrophoresis and was found within the density range between 1.006-1.02 g/ml, yet HDL_c lacked apo B. Moreover, HDL lipoproteins of $alpha_1$ mobility and arginine rich apolipoprotein decreased in

the density 1.08-1.210 g/ml fraction while HDL_c increased, when rats were fed high cholesterol diets. LDL and VLDL became the principle cholesterol carrying rat plasma lipoproteins after dietary induced hypercholesterolemia (Mahley et al., 1977a).

A lipoprotein designated HDL_1 was found by ultracentrifugally fractionating normal rat plasma in the density between 1.02-1.063 g/ml. In addition, HDL_1 was separable from LDL by Geon Pevikon electrophoresis. HDL_1 was found to be the normal rat's equivalent to HDL_c present in the hypercholesterolemic rat (Weisgraber et al., 1977). HDL_1 has a high arginine rich apolipoprotein content which when injected into normal rats finds its way to HDL, but in hypercholesterolemic rats is more predominant in beta VLDL, LDL and HDL_c. HDL_c production following high dietary cholesterol feeding has been demonstrated in other species besides the rat, including the dog and human (Mahley, 1978).

III. INTRAVASCULAR TRANSFER OF LIPOPROTEIN LIPIDS

A. Lipoprotein-Unesterified Cholesterol Transfer

1. Equilibration

Unesterified cholesterol was found to equilibrate between plasma lipoproteins during an in vitro 37 °C incubation within 2-5 hours, which was observed using incubations of rat plasma perfused through the liver. However, unesterified cholesterol would not equilibrate between plasma lipoproteins when rat plasma was incubated at 4 °C. Moreover, unesterified cholesterol was found to exchange between all rat plasma lipoproteins as did triglycerides and phospholipids, but rat plasma esterified

cholesterol was not observed to exchange between plasma lipoprotein. (Roheim et al., 1963). Human cord blood incubated at 37 °C with HDL and LDL as the only lipoproteins present, demonstrated a net mass transfer of unesterified cholesterol from LDL to HDL (Sniderman et al., 1978).

2. LCAT's Relationship to Unesterified Cholesterol Transfer

Net transfer of unesterified cholesterol between plasma lipoproteins does not appear to occur when LCAT is inhibited (Fielding et al., 1981a; Perret et al., 1983), yet a time lag of 2-5 hours is required for unesterified cholesterol concentrations to equilibrate between the lipoproteins. The transfer of unesterified cholesterol to HDL is probably due to LCAT activity creating an unsaturated HDL particle with respect to unesterified cholesterol, allowing more unesterified cholesterol to accumulate on the HDL particle (Perret et al., 1983). Therefore, the time lag for equilibration of unesterified cholesterol between plasma lipoproteins incubated in vitro when LCAT was inhibited must reflect the time it would take for the relatively unsaturated HDL particles to reach saturation with respect to unesterified cholesterol. The transfer of unesterified cholesterol is believed to be a passive process whether between plasma lipoproteins or between cells and lipoproteins (Glomset et al., 1966; 1973; Phillips et al., 1980), however apo A-I the major apolipoprotein component of HDL appears to promote sterol efflux from the cell surface of fibroblasts during an in vitro incubation (Fielding et al., 1981b). The combination of HDL being the preferred substrate for LCAT activity and apo A-I promoting sterol efflux could account for

the passive transfer of unesterified cholesterol to the HDL molecule *in vitro* as well as *in vivo*, when LCAT is active.

B. Lipoprotein-Cholesteryl Ester Transfer

1. Cholesteryl Ester Transfer and Exchange

Rat plasma cholesteryl ester turnover was found to be dependent on the fatty acid moiety and the lipoprotein class, unlike human plasma where cholesteryl ester turnover is similar for all cholesteryl esters. This is due to the marked heterogeneity between cholesteryl esters of all rat lipoproteins as compared to the homogeneity of human plasma lipoprotein-cholesteryl esters (Gidez *et al.*, 1967). An *in vivo* study on humans performed using labeled mevelonate suggested a process exists by which cholesteryl esters equilibrate between plasma lipoproteins. However, some heterogeneity was found to exist between the cholesteryl ester composition of each human plasma lipoprotein class, indicating that the same mechanism operates to equilibrate the cholesteryl ester content of human plasma lipoprotein fractions, but at a different rate (Goodman, 1964). Unesterified cholesterol and phospholipid decreased with a concomitant increase in cholesteryl esters after a 36 hour *in vitro* incubation of human plasma. In addition, triglycerides increased in HDL while cholesteryl ester decreased during the incubation suggesting that a transfer and exchange of cholesteryl ester for triglycerides occurred between other lipoproteins and HDL (Rehnborg *et al.*, 1964). Moreover, the cholesteryl ester content of human plasma VLDL increased,

while the cholesteryl ester content of LDL and HDL decreased after a 16 hour incubation at 37 °C when LCAT was inhibited. At the same time, VLDL triglycerides were shown to decrease, while LDL and HDL triglycerides increased (Nichols et al., 1965).

An exchange of cholesteryl esters between HDL and LDL was demonstrated to occur when human cord blood was fractionated and incubated with HDL and LDL alone, but no evidence of cholesteryl ester transfer was observed. Cholesteryl ester exchange was determined by the movement of labeled cholesteryl ester from one lipoprotein to another without a concomitant change in cholesteryl ester concentration, while cholesteryl ester transfer was determined by changes in lipoprotein-cholesteryl ester concentration. However, VLDL was not included in the incubation medium of this study limiting the ability to determine whether lipoprotein-cholesteryl ester transfer could occur between HDL and VLDL (Sniderman et al., 1978). In contrast, net transfer of cholesteryl esters from HDL to VLDL was found when tritiated cholesterol dissolved in HDL was administered to rabbits in vivo, while lipoprotein-cholesteryl ester exchange was not observed to occur (Barter et al., 1979b). The sources for human plasma VLDL cholesteryl esters were established to be both the liver and the plasma in humans (Barter, 1974a; 1974b), then it was determined that the plasma source of VLDL cholesteryl esters was HDL (Barter, 1976).

It was established by using ultracentrifugal techniques coupled with radioisotopic labeling of cholesteryl esters that rat plasma lacked significant cholesteryl ester transfer activity as compared to other

species (Barter et al., 1978). Many species appear to have the ability to transfer esterified cholesterol between plasma lipoproteins with the exceptions being the rat, dog and pig (Ha et al., 1982). The reported lack of cholesteryl ester transfer activity in rat plasma could explain the heterogeneity found between the cholesteryl ester composition of the rat's plasma lipoproteins as compared to the homogeneous cholesteryl ester composition of human plasma lipoproteins due to the exchange and transfer of cholesteryl esters between lipoproteins. Since VLDL is synthesized in the liver the cholesteryl ester composition of rat plasma VLDL is determined by the liver and plasma HDL-cholesteryl ester composition is determined by plasma LCAT activity.

2. Lipid Transfer Factor

Due to the lack of lipoprotein-cholesteryl ester transfer in some species and not in others and to the energy requirements needed to remove cholesteryl ester from the core of the lipoprotein molecule, a plasma transfer factor was postulated to exist, which could perform the plasma lipoprotein-cholesteryl ester transfer process (Goodman, 1964; Nichols et al., 1965). The rabbit fed a high cholesterol diet was found to have a plasma fraction of density greater than 1.25 g/ml which could stimulate plasma lipoprotein-cholesteryl ester transfer or exchange and LCAT activity was proportional to lipoprotein-cholesteryl ester transfer, although cholesteryl ester transfer still occurred when LCAT was inhibited (Zilversmit et al., 1975). Moreover, a cholesteryl ester exchange protein was isolated in the density greater than 1.210 g/ml plasma fraction of rabbit plasma which could be demonstrated to exchange

labeled cholesteryl ester between rabbit LDL and bovine HDL. In contrast to the density greater than 1.25 g/ml plasma fraction, the plasma fraction denser than 1.210 g/ml seemed only responsible for exchange of cholesteryl ester between LDL and HDL, while LCAT had no relationship to exchange activity (Pattnaik et al., 1978).

The possibility arose that a previously described apolipoprotein, apo D, might be the cholesteryl ester transfer protein, since the transfer protein isolated by one group of investigators seemed to have the same isolation and electrophoretic characteristics as apo D (Chajek et al., 1978). However, a more purified lipid transfer protein did not have the characteristics of any previously described apolipoprotein, including apo D (Morton et al., 1982). A transfer complex isolated from human plasma had HDL substrate specificity, but it could not be established whether this complex which contained LCAT and HDL was an artifact of ultracentrifugation (Fielding et al., 1980). However, it has been shown that HDL_3 and VHDL incubated with LCAT supply cholesteryl esters for transfer to VLDL. Furthermore, it was suggested that an HDL_3/LCAT complex exists producing by LCAT activity a smaller lipoprotein which may be transferred to acceptor lipoproteins which could account for lipoprotein-cholesteryl ester transfer (Rose et al., 1982).

Plasma LCAT activity, although not necessary for lipoprotein-cholesteryl ester exchange was proportional to cholesteryl ester transfer activity in the hypercholesterolemic rabbit. Therefore, the more cholesteryl ester present in plasma the more exchange or transfer of cholesteryl ester between lipoproteins occurred (Zilversmit et al., 1975).

plasma lipoprotein-cholesteryl ester transfer from HDL to VLDL and chylomicrons was demonstrated when LCAT was inhibited, while no cholesteryl ester transfer could be observed from HDL to LDL (Marcel et al., 1981). However when LCAT activity was present, a net mass transfer of cholesteryl ester from HDL to LDL and VLDL occurred, which was only limited by the concentration of the cholesteryl ester acceptors, LDL and VLDL, present in the incubation medium (Chajek et al., 1978). Since LDL was recently shown to support a limited amount of LCAT activity (Barter, 1983), this may account for the observed transfer of cholesteryl ester from HDL to LDL, because esterification of unesterified cholesterol and its incorporation into LDL may appear to be transfer if LCAT is not inhibited. Transfer of plasma lipoprotein-cholesteryl esters from HDL to VLDL appeared to be more rapid than cholesterol esterification by LCAT in rabbit plasma. However, lipoprotein-cholesteryl ester transfer or exchange in human plasma occurs at about the same rate as cholesterol esterification (Nestel et al., 1979). Moreover, HDL_3 the subfraction for which LCAT is most specific in human plasma (Fielding et al., 1972a), appears to have the most lipoprotein-cholesteryl ester transfer activity (Groener et al., 1984). In addition, the cholesteryl ester content of VLDL and chylomicrons could be due to the activity of LCAT and lipoprotein-cholesteryl ester transfer activity in those species which have a lipoprotein-cholesteryl ester transfer factor (Marcel et al., 1980). Therefore, the evidence indicates that the ultimate source of cholesteryl esters for transfer in the human is LCAT (Schwartz, et al., 1982).

Cholesteryl ester exchange has been observed between lipoproteins and phospholipid vesicles, but only between HDL and the vesicles, since LDL and VLDL could not promote exchange, when incubated alone with the phospholipid vesicles (Pattnaik et al., 1979). Cholesteryl ester transfer from HDL to vesicles was found to require the density greater than 1.210 g/ml fraction of either human or rabbit serum and cholesteryl ester transfer was also found to occur with either HDL_1, HDL_2 or HDL_3 (Young et al., 1981). LDL and VLDL incubated together appeared to exchange cholesteryl ester between each other, but no transfer of cholesteryl esters was observed (Chajek et al., 1978). In contrast, another study demonstrated that lipoprotein-cholesteryl ester transfer may occur between LDL and VLDL (Barter et al., 1980). The probability of a transfer protein picking up esterified cholesterol in human plasma for HDL versus VLDL versus LDL is 28.9:4.65:1 (Barter et al., 1982b), which would explain the preference for obtaining cholesteryl ester for transfer from HDL (Chajek et al., 1980). Moreover, this would suggest that the lipoprotein-cholesteryl ester transfer protein may be nonspecific (Barter et al., 1982a).

C. Lipoprotein-Triglyceride Transfer

Exchange of cholesteryl ester for triglyceride between lipoproteins was first demonstrated in human plasma samples incubated without LCAT inhibition (Rehnborg et al., 1964). Further investigation indicated that lipoprotein-triglyceride and cholesteryl ester transfer may be independent since lipoprotein triglyceride transfer can be inhibited by thiol group blockade, whereas lipoprotein-cholesteryl ester transfer

is not (Hopkins et al., 1980; 1982). However, using purified lipid transfer protein isolated from human plasma, both lipoprotein-cholesteryl ester and triglyceride transfer could be demonstrated (Morton et al., 1982). Moreover, others believe that lipoprotein-triglyceride and cholesteryl ester transfer may be linked to phospholipid transfer or exchange (Ihm et al., 1980; 1982). Whether a single factor or lipid transfer complex exists for the transfer of both lipoprotein-cholesterol and triglyceride remains to be established. Although early studies have shown that a lipoprotein-triglyceride exchange occurs between rat lipoproteins (Roheim et al., 1963), no evidence of significant triglyceride transfer could be observed in rat plasma as compared to human and rabbit plasma (Barter et al., 1979a; 1979d).

D. Lipoprotein-Phospholipid Transfer

Phospholipid exchange was demonstrated during in vitro incubations of rat plasma (Roheim et al., 1963). Exchange of phospholipid for unesterified cholesterol was also observed between rat chylomicrons and human HDL by what was postulated to be a passive process, initiated by collisions between the two particles (Lippiello et al., 1983). In contrast, exchange of phosphatidyl choline was found to be enhanced by a partially purified phospholipid transfer factor as was shown by an in vitro incubation of unilamellar liposomes with human plasma HDL (Damen et al., 1982). HDL has been shown to have a high capacity for phosphatidylcholine uptake and that membrane transfer can occur from phosphatidylcholine vesicles to HDL (Jonas, 1979), however with the addition of a density greater than 1.21 g/ml fraction of plasma the phosphatidyl cho-

line transfer to HDL was markedly enhanced possibly due to alteration in the membrane, facilitating transfer (Tall et al., 1983b). Proteins facilitating the transfer of phosphatidyl choline and cholesteryl ester from HDL to LDL were partially purified from human plasma and the protein or proteins responsible for both transfer processes coeluted at every purification step. In addition, the rat was found to have a protein, which facilitated lipoprotein-phospholipid transfer, but not lipoprotein-cholesteryl ester transfer indicating that the two processes may be handled by at least two different proteins (Ihm et al., 1980). This was confirmed, when two protein fractions were isolated from human plasma each having the separate ability of either lipoprotein-cholesteryl ester or phospholipid transfer (Tall et al., 1983a). It has been suggested that the lipoprotein-phospholipid transfer factor exists in a complex with LCAT and lipoprotein-cholesteryl ester exchange protein in human as well as in rat plasma (Ihm et al., 1982), even though rat plasma has very little lipoprotein-cholesteryl ester transfer activity (Barter et al., 1978). This would be consistent with evidence that the rat may have a plasma substance which inhibits the lipoprotein-cholesteryl ester transfer factor (Morton et al., 1981).

E. Rat Lipoprotein-Cholesteryl Ester Transfer

The transfer of cholesteryl ester between HDL and lower density lipoproteins has previously been reported to occur in rat plasma at extremely low rates when compared to other species such as rabbits and humans (Barter et al., 1978; 1979b; 1979c; Oschry et al., 1982). A plasma inhibitor to the lipoprotein-cholesteryl ester transfer factor

has been demonstrated in the rat which may account for the low rates of lipoprotein-cholesteryl ester transfer observed between rat plasma HDL and VLDL (Morton et al., 1981). Recently, two HDL subfractions that were isolated from rat plasma by HDL gradient ultracentrifugation were found to display cholesteryl ester transfer activity employing an in vitro assay. Rat plasma cholesteryl ester appeared to be transferred from a less dense HDL subfraction to a denser HDL subfraction, both isolated by density gradient ultracentrifugation (Jansen et al., 1983). In contrast, two rat plasma HDL subfractions isolated by zonal ultracentrifugation, did not show any cholesteryl ester transfer activity in vitro. However, in vivo, transfer of cholesteryl ester between these two HDL subfractions was observed, with the transfer occurring from the denser HDL subfraction to the less dense HDL subfraction when the denser HDL subfraction containing radioactively-labeled cholesterol was administered to rats (Eisenberg et al., 1984). The conflicting observations of these two reports regarding cholesteryl ester transfer between various rat plasma HDL subfractions may be due to differences in lipoprotein compositions resulting from separation of lipoprotein fractions employing ultracentrifugal techniques (Lindgren et al., 1979).

IV. ESTROGENIC REGULATION OF LIPOPROTEIN RECEPTORS

A. Hypolipidemic Effect of Estradiol on Rats

1. Hypocholesterolemia and Lipoprotein Clearance

In vivo administration of 17 α-ethinyl estradiol to rats has produced marked hypocholesterolemia. Estrogen treatment lowered plasma cholesterol levels by 90%, while plasma apolipoproteins were lowered as well (Davis et al., 1978). Estrogen treatment was found to cause a complete disappearance of LDL in rat plasma. Moreover, the liver was the organ responsible for the uptake and degradation of LDL in estrogen treated animals as was demonstrated by injecting labeled LDL into rats treated with estrogen (Hay et al., 1971). This was confirmed when the rate of LDL clearance was found to decrease in functionally eviscerated rats as compared to controls, when prior to evisceration both groups had augmented LDL clearance due to ethinyl estradiol treatment (Chao et al., 1979).

2. Specific Lipoprotein Receptor Induction

LDL uptake by rat liver in vivo appeared to resemble the uptake seen in vitro by specific LDL receptors found in fibroblasts, since the liver membranes removed from ethinyl estradiol treated rats showed a marked binding preference for LDL over HDL, required calcium for binding, failed to bind LDL which had been modified and the binding capability was destroyed by pronase, all characteristics of the classical LDL binding receptor observed in fibroblasts (Kovanen et al., 1979b). Further characterization of the binding properties of the liver membranes of ethinyl estradiol treated rats revealed a specificity for apo B and apo E containing lipoproteins (Windler et al., 1980b). Conversely

female rats, which were ovariectomized showed a marked increase in plasma cholesterol, triglycerides and apolipoproteins B, E and C. The main reason for the increase was found to be due to an increase in LDL and HDL in the ovariectomized rat (Van Lenten et al., 1983). The increases in those lipoproteins were probably due to the decrease in uptake of apo E containing HDL and apo B containing LDL by lipoprotein receptors which were reduced, because of the loss of estrogen stimulated production.

B. Sex Hormones and Human Lipoprotein Catabolism

1. Oral Contraceptives and Estrogen Replacement

It has long been known that premenopausal women have a lower risk of developing coronary heart disease than men and that risk increases to the same as men after menopause (Barr et al., 1955). Since estrogen appears to produce a marked hypocholesterolemia in rats, it is interesting to postulate a protective role for estrogen in the human female by increasing lipoprotein-cholesterol clearance and reducing the risk of coronary heart disease. Studies on women given estrogen replacement therapy following menopause and on women given oral contraceptives have yielded information on the influence of sex hormones on lipoprotein metabolism. Premenopausal women have higher plasma HDL levels than men (Levy et al., 1966) and plasma HDL has been inversely correlated with the risk of developing coronary artery disease (Gordon et al., 1977; see section V.C.3. HDL-cholesterol). Women on estrogen therapy have

increased HDL levels (Cauley et al., 1983)., but women on combined estrogen-progesterone therapy do not (Cheung et al., 1977). A reduced plasma HDL level was found in women taking contraceptives (Arntzenius et al., 1978), which may be due to progesterone content, since it was found that HDL increases with the amount of estrogen and decreases with the amount or potency of progesterone administered (Bradley et al., 1978; Farish et al., 1983). Elevated plasma apo A-I values occur in humans with high plasma estrogen levels, whether the estrogen is from exogenous or endogenous sources (Albers et al., 1976). Myocardial infarction risk however, increases with the use of oral contraceptives and that increased risk has been found to continue after contraceptive use ceases in long term users (Slone et al., 1981). Women taking oral contraceptives were found to have a death rate 40% greater than those who had never taken them and the excess deaths were found to be due to circulatory system disease, mostly from excessive clotting (Royal College of General Practitioners, 1981). Moreover, lowering the estrogen content of contraceptive pills was found to lower the incidence of thromboembolic disease (Bottiger et al., 1980a).

2. Estrogen and Male Susceptibility to Myocardial Infarction

While estrogens may have a protective effect toward coronary artery disease in women possibly by increasing their HDL-cholesterol levels, it has been found that men with myocardial infarction have higher plasma estrogen levels than those who do not have an infarction (Phillips et al., 1983). In contrast, testosterone was found to have a

significant positive correlation with plasma HDL in adult men (Heller et al., 1981b), yet no significant correlation could be found between testosterone or estrogen and male patients who had previous myocardial infarctions (Heller et al., 1981a). Unfortunately, this study was not controlled for drug usage nor time following the infarction, since some patients included in the study had their infarction eleven years prior to the time their steroid hormones were evaluated. Another year long study on adolescent boys determined an increase in plasma testosterone occurred with a decrease in plasma estrogen, which corresponded to an increase in plasma triglycerides and LDL-cholesterol, while HDL-cholesterol decreased (Laskarzewski et al., 1983). The lipid changes however, may be an age related phenomenon unassociated with the change in hormone levels.

The cause of estrogen's protective nature regarding myocardial infarction in women and the increased risk in men is unknown at present. A recent research news report in Science magazine (Kolata, 1983) received four different opinions of this problem from four researchers. One postulated that estrogen increased the development of atherosclerosis, which seems unlikely due to the decrease in risk of coronary artery disease associated with the raising of HDL-cholesterol levels as seen in women taking estrogen contraceptives (Cauley et al., 1983). Another postulates that estrogen increases blood clotting, which would agree with results seen in the contraceptive studies (Bottiger et al., 1980a; Royal College of General Practitioners, 1981). The other two researchers feel that estrogens either increase the work load of the heart or cause

coronary vasospasms leading to myocardial infarction.

V. ATHEROSCLEROSIS

A. Arteriosclerosis

1. Definition

Arteriosclerosis is a disease of blood vessels characterized by a thickening and hardening of the blood vessel walls with a loss of elasticity, which occurs most frequently in arteries. Atherosclerosis is the most common form of arteriosclerosis, characterized by the localized accumulations of lipid containing material within or beneath the intimal surfaces of blood vessels.

2. Development

Atherosclerosis is believed to progress from fatty streaks, which occur in childhood resulting from the deposition of lipids mostly in the form of unesterified cholesterol or cholesterol oleate in the smooth-muscle cells and macrophages of the arterial intima. An increased number of smooth-muscle cells and an increased amount of connective tissue surround and cover the deposit of cholesterol-rich lipid in the lining of the lumen of the artery. The connective tissue and smooth muscle cells surrounding the cholesterol-rich lipid core is known as a fibrous plaque, however not all fatty streaks develop into fibrous plaques and there is considerable controversy as to whether the fatty streak is actually the initial condition from which the fibrous plaque develops. As atherosclerosis progresses, the fibrous plaque enlarges and undergoes

complex changes, becoming vascularized from both the lumen and the media. The fibrous plaque's lipid core also increases in size and becomes calcified. The intimal surface may disintegrate and ulcerate or the blood vessels may rupture and hemorrhage into the plaque. The ultimate result of the atherosclerotic process is that the lumen of the blood vessel becomes blocked, decreasing blood flow to the tissue the vessel supplies (Report of the Working Group on Arteriosclerosis, 1981).

B. Theories of Atherosclerotic Development

1. Lipid Infiltration Hypothesis

The lipid infiltration hypothesis suggests that lipids filter through the endothelial lining of the artery and accumulate in the intima of the artery. High plasma lipid levels would therefore cause an increase in the passive diffusion of lipid between the junctions of the endothelial cells with a subsequent increase in the lipid concentration of the arterial intima. Plasma proteins, including lipoproteins have been demonstrated to penetrate the intact endothelium, therefore lipoprotein lipid could be deposited within the intima and initiate the atherosclerotic process (Bratzler et al., 1977; Reichl et al., 1973). Moreover, the theory is supported by the experimental evidence that atherosclerotic lesions can be induced by feeding high fat, high cholesterol diets to experimental animals (Camejo et al., 1973; Kramsch et al., 1981; Mahley et al., 1974; Mahley, 1978; Shore et al., 1974), since high cholesterol diets increase the plasma cholesterol levels of certain

animal species promoting the deposit of lipid within the intima of arteries. This hypothesis however, does not explain why atherosclerotic lesions do not appear at random, but appear more frequently at areas of stress in the arterial tree, particularly within the coronary arteries (Ross, 1979).

2. Response to Injury Hypothesis

The response to injury hypothesis suggests that some form of injury occurs to the endothelial cells lining the lumen of the artery. The protective endothelial barrier could be altered in some way as to increase permeability toward lipoproteins and lipids, which in part agrees with the lipid infiltration hypothesis, but assumes an underlying cause for the increase in lipid permeability. Alternatively, the postulated damage could be so severe as to remove the endothelial cells and cause platelet aggregation at the site of injury. Platelet release of substances including platelet derived growth factor (PDGF), would cause the focal migration of smooth muscle cells to the site of injury and their subsequent proliferation. Plasma lipid carried by lipoproteins, particularly LDL would have easier access to the area affected and lipid accumulation would ensue. Chronic injury to the area would promote the progression of the lesion and if the factor leading to injury was temporary the process could be reversed and the lesion regress (Ross, 1979). No direct evidence has demonstrated that initiation of the atherosclerotic lesion is due to loss of the endothelial lining, however experimentally induced atherosclerotic lesions have been produced by damaging the

endothelial lining mechanically, by irradiation, or by changes in temperature, pH and osmolarity. Other factors implicated as causes for damaging the endothelial tissue in humans are endotoxins, hyperlipidemia, antigen-antibody complexes, carbon monoxide, viral infection and hemodynamic stress (Grundy, 1983). Hypertension has also been implicated as a factor increasing the risk of developing atherosclerosis in humans. Experimental models of hypertension have demonstrated intracellular enzymatic changes affecting cholesterol metabolism, therefore the initial injury caused by undue vessel stress due to hypertension could lead to enzyme modification of lipid metabolism, increasing deposition of cholesteryl esters within the cell and initiating the atherosclerotic process (Hayashi et al., 1982).

3. Monoclonal Hypothesis

The monoclonal hypothesis assumes that atherosclerosis is a proliferative disease. The hypothesis suggests that the proliferative smooth muscle cells in the atherosclerotic lesions are derived from a single progenitor cell, therefore the lesions are similar to a benign neoplasm (Ross, 1982). The hypothesis is based on the observation that a single isoenzyme of glucose-6-phosphate dehydrogenase EC 1.1.1.49 (Nomenclature Committee, 1979) was present in lesioned arteries, whereas normal arteries had a mixture of the two isoenzymes (Benditt et al., 1973). This does not necessarily mean that the lesion arises from one cell, but could mean that one cell type is more proliferative than the other.

C. Lipoproteins and Atherosclerosis

1. VLDL and Triglyceride

The atherogeneity of VLDL the major triglyceride containing lipoprotein of human plasma is as yet unresolved (Grundy, 1983; Riemersma, 1984). The results of epidemiological studies determining whether triglyceride and VLDL levels correlated to the presence or severity of atherosclerotic disease have been inconsistent. A recent study has shown a greater amount of triglycerides and VLDL-triglycerides in patients suffering from coronary artery disease as compared to normal age matched controls. A fat tolerance test performed on all subjects in the study indicated the patients with coronary artery disease cleared triglycerides from the plasma slower than the normal control subjects (Tollin et al., 1984). The study was undertaken to confirm a previous project, which had demonstrated a positive correlation between serum triglyceride concentration and the presence of coronary artery disease (Bottiger et al., 1980b). Moreover, VLDL-cholesterol concentration has been shown to be a good predictor of coronary artery disease in humans over 50 years of age (Whayne et al., 1981).

Although many studies have shown that patients with coronary artery disease tend to have high plasma triglyceride levels, when other factors were considered the significance of high serum triglycerides as a predictor of the risk of obtaining coronary artery disease disappeared. The Framingham study demonstrated upon univariate analysis of the data that serum triglyceride was significantly correlated with coro-

nary artery disease in women, however using multivariate analysis no significant correlation was found (Gordon et al., 1977). Serum triglycerides as determined in the Framingham study were not consistently found to be a significant factor in coronary heart disease risk (Castelli et al., 1977). Apolipoprotein B, a major component of VLDL as well as LDL was determined in a human population, which had suffered from previous myocardial infarction and compared to a human population with hypertriglyceridemia. Higher apo B concentrations were found in those patients with previous myocardial infarction and in the hypertriglyceridemia patients with symptoms of coronary artery disease, than in patients with hypertriglyceridemia and no symptoms of coronary artery disease. Serum triglycerides although high in both populations, were not as important predictors of coronary artery disease as the apo B concentration (Sniderman et al., 1982).

Studies attempting to link serum triglyceride levels to the severity of coronary artery disease in humans have also suffered from inconsistencies. VLDL-triglyceride has been found to be significantly correlated with the severity of coronary artery disease, while serum triglyceride results were inconclusive (Jenkins et al., 1978). Other studies have indicated that serum triglycerides have no discriminating function at all as to severity of coronary artery disease (Noma et al., 1983; Riesen et al., 1980; Vander Heiden et al., 1984).

HDL-cholesterol, which will be discussed shortly has been negatively correlated with coronary artery disease (Gordon et al., 1977) and has been negatively correlated with VLDL-triglyceride concentration

(Gordon et al., 1977; Miller et al., 1975; Schaeffer et al., 1978a). Hypertension, a primary risk factor for development of atherosclerotic disease is related to body mass as is plasma triglyceride concentration. Moreover, epidemiological studies indicate that triglyceride levels appear to be related to cholesterol concentration. Therefore, plasma triglycerides are related to factors which are in turn related to the risk in humans of developing atherosclerotic disease, but plasma triglyceride levels alone are not necessarily related to coronary artery disease. No persuasive evidence could be found in the Western Collaborative Group study or in a twenty year review of published epidemiological reports that triglycerides are involved in coronary heart disease (Hulley et al., 1980). On the other hand, a mechanism has been proposed whereby VLDL could be involved in the atherosclerotic process. Zilversmit (1973) has suggested that lipoprotein lipase by its location on the cell surface and its actions on VLDL creates a lipoprotein particle with a high cholesterol content in close proximity to the cell surface, thus increasing the likelihood of cholesterol being taken up into the intima. It has also been suggested that the liberation of free fatty acids by lipoprotein lipase's action on VLDL triglycerides creates a disturbance in the endothelial lining increasing the permeability of the artery, allowing LDL or the newly formed VLDL remnant ready access to the intimal layer of the artery (Hulsmann et al., 1975).

The epidemiological evidence thus far accumulated appears to indicate that serum triglycerides and VLDL triglycerides may not play a primary role in the induction of atherosclerotic lesions. However, epide-

miological studies are based on lipid levels determined at a particular time on a particular day and are unrelated to processes involved in actual lipid metabolism. VLDL may play an important secondary role in atherogenesis as the precursor to LDL (Eisenberg et al., 1973a) or by VLDL's inverse relationship to HDL (Miller et al., 1975). VLDL's contribution to the generation of atherosclerotic disease may not be clearly understood until consideration is given to the plasma lipid metabolic processes, which can not be determined in epidemiologic studies.

2. LDL and Cholesterol

Recently the atherogeneity of human plasma total cholesterol has been demonstrated by the Lipid Research Clinics Program (1984a; 1984b). The program had completed a study showing that drug therapy which decreased plasma total cholesterol concentrations in patients suffering from hypercholesterolemia was responsible for a decrease in the incidence of coronary heart disease and coronary artery disease. The plasma total cholesterol was found to be directly proportional to the LDL-cholesterol level. Since LDL is the major cholesterol carrying lipoprotein in the human, the reduction in plasma total cholesterol found in this study was found to be due to a reduction in plasma LDL-cholesterol. This study has confirmed what has been implied since the 1950's, that LDL elevation in human plasma was strongly correlated with atherosclerosis (Gofman et al., 1954).

Epidemiological studies have shown in almost all cases a positive correlation between the risk of developing either coronary heart disease or coronary artery disease and the plasma concentration of LDL-choles-

terol. The Framingham study (Gordon et al., 1977; Castelli et al., 1977) had found significant positive correlations between LDL-cholesterol and coronary heart disease, but the Framingham study when adjusted for age did not find a significant relationship between plasma total cholesterol and coronary heart disease, as was also true for the Stockholm study (Bottiger et al., 1980b).

Although LDL-cholesterol has been found to be a significant predictor of coronary heart disease, determination of human plasma LDL-cholesterol concentration may be an even better predictor of coronary artery disease severity. Plasma LDL-cholesterol and apo B concentrations have been found to be the best predictors of human coronary artery disease severity in several studies (Noma et al., 1983; Riesen et al., 1980; Sniderman et al., 1982; Vander Heiden et al., 1984). All of these studies demonstrated a positive correlation between plasma LDL-cholesterol concentration and apo B concentration and the degree of angiographically measured coronary heart disease. Moreover, the apo B concentration appeared to be the best discriminator of coronary disease severity, particularly in a study, which used subjects who had plasma total cholesterol levels below 265 mg/dl (Whayne et al., 1981).

Since LDL carries 70% of human total plasma cholesterol and atherosclerotic disease is due to the deposit of cholesteryl esters within the arterial wall it has been assumed that LDL-cholesterol is the primary lipoprotein involved in the human atherogenic process. The LDL receptor pathway however, as described by Goldstein and Brown (1977a) is a nonatherogenic pathway, because LDL receptors are down regulated by

cholesterol accumulation preventing cells from taking up more cholesterol than they need (see section II.C.4. LDL Catabolism). Yet, atherosclerosis has been found to occur when human plasma LDL-cholesterol concentration increases due to either inherited lipoprotein receptor defects or environmental factors. The increased LDL-cholesterol may be taken up in cells by phagocytosis via the scavenger cell pathway, which is not regulated by cholesterol uptake, thus cholesterol can accumulate in the scavenger cells to a tremendous extent forming the foam cells seen in atherosclerotic lesions (Brown et al., 1980; Goldstein et al., 1977b; Packard et al., 1983).

Clearly epidemiological and experimental evidence supports the atherogenic potential of LDL-cholesterol, although the epidemiological evidence for plasma total cholesterol as a risk factor for atherogenesis is not as convincing. One area of LDL and atherosclerotic research which needs to be addressed on the molecular level is the phagocytosis of unmodified LDL (see section II.C.4. LDL Catabolism), since the scavenger pathway does not appear to recognize normal LDL, but only a modified more electronegative LDL (Brown et al., 1980). Therefore, in vivo LDL modification prior to phagocytosis has yet to be completely understood. Moreover, the relationship of other risk factors to atherogenesis and LDL metabolism must also be explored to determine what LDL's function is in atherosclerotic lesion production.

3. HDL-cholesterol

Many biological systems have balances, like feedback inhibition, to prevent excesses from occurring. LDL, which appears to be an atherogenic lipoprotein would hopefully be balanced by a nonatherogenic, protective lipoprotein. Studies have indicated that HDL may be that lipoprotein. A relationship between alpha$_1$ lipoprotein-cholesterol (HDL) and reduced risk of obtaining atherosclerosis was demonstrated in the 1950's (Barr, 1951). It was also noted that a high plasma total cholesterol concentration was related to a high plasma beta lipoprotein-cholesterol (LDL) concentration but low alpha$_1$ lipoprotein-cholesterol concentration was a more consistent predictor of atherosclerotic disease. However, it was not until 1975 that interest was rekindled in alpha$_1$ lipoprotein, when a study using subjects with plasma cholesterol levels less than 250 mg/dl found that the HDL-cholesterol levels were significantly lower in those patients with coronary artery disease than those without coronary artery disease (Miller et al., 1975). Epidemiological studies have consistently shown that HDL-cholesterol is negatively correlated to the risk of obtaining coronary heart disease (Castelli et al., 1977; Gordon et al., 1977). Moreover, the major apolipoprotein of HDL, apo A-I may be an even better discriminator of coronary artery disease than HDL-cholesterol (Maciejko, et al., 1983).

Despite HDL-cholesterol's discriminant power in predicting the incidence of atherosclerotic disease, HDL-cholesterol does not appear to be a good predictor of the severity of atherosclerotic disease (Noma et al., 1983). Apo A-I, apo B, apo A-II, TC and LDL-C were found to be

good discriminators of severity of coronary artery disease, but HDL-cholesterol was not (Riesen et al., 1980). Interestingly, apo A-I and HDL-cholesterol have been found to be very closely correlated (Maciejko et al., 1983), therefore the discriminating power of apo A-I as compared to HDL-cholesterol should be similar, which was not indicated in the study by Riesen and coworkers. Moreover, another study has demonstrated that HDL-cholesterol concentration is a good discriminator of atherosclerotic disease severity (Jenkins et al., 1978), therefore the discriminatory power of HDL-cholesterol as a predictor of atherosclerotic disease severity is still controversial.

Virtually every epidemiological study demonstrates a negative correlation between human plasma HDL-cholesterol concentration and coronary artery disease or coronary heart disease. People involved in aerobic exercise have a lower incidence of coronary heart disease than the rest of the population and more HDL-cholesterol than sedentary people or those who exercise anaerobically (Clarkson et al., 1981; Hartung et al., 1980). People in areas free of coronary heart disease have higher HDL-cholesterol concentrations than people in areas of high incidence for coronary heart disease (Walker et al., 1978). Consumption of alcohol has been shown to be associated with a decreased risk of obtaining coronary heart disease (Dyer et al., 1980; Klatsky et al., 1974). Moreover, alcohol consumption has been associated with an increase in plasma HDL-cholesterol concentration (Haskell et al., 1984; Thornton et al., 1983). Although these studies do not necessarily imply a direct link between HDL-cholesterol and atherosclerotic risk, they are suggestive that HDL

plays an important role in the prevention of atherosclerotic disease.

The protection HDL-cholesterol appears to provide from atherosclerosis has been postulated to be due to HDL acting as a cholesterol scavenger (see section II.C.7. HDL Catabolism). In vitro studies have shown HDL is able to attract the unesterified cholesterol from tissue membranes (Fielding et al., 1981b). LCAT for which HDL has been demonstrated to be the preferred substrate (Fielding et al., 1971), esterifies cholesterol on the surface of the HDL molecule and the esterified cholesterol is stored in the hydrophobic core of the HDL molecule allowing room for the accumulation of more unesterified cholesterol on the surface (Glomset et al., 1973). Cholesterol has been postulated to be cleared from the cells by this mechanism, although no direct in vivo evidence has proven this. Removal of cholesterol from human plasma may be dependent on HDL uptake by the liver or more probably by cholesteryl ester transfer to lower density lipoproteins (see section III.B.1. Cholesteryl Ester Transfer and Exchange) which ultimately results in the increase of cholesteryl esters within LDL, which are then removed through the LDL receptor pathway (Brown et al., 1981).

Presently, the factors which are known and can be determined to account for the risk in humans of developing coronary artery disease may be able to predict the disease only 50% of the time (Crouse, 1984). The focus of the search for better predictors of coronary artery disease is on the constitutive fractions of lipoproteins and coagulation factors leading to thrombi, which can block the arteries. Other possible factors including dietary and psychological studies are also being

explored. Lipoprotein concentrations have limited value as a diagnostic tool in those patients showing symptoms of coronary artery disease, but the great value may come as a predictive index for the potential of developing such disease and the continued elaboration of the functional roles of lipoproteins will lead to the ultimate understanding of the atherosclerotic disease process and its cure (Blackburn, 1983).

D. Species Susceptibility to Atherosclerosis

1. Rat Atherosclerosis

Proliferative lesions have been found to occur in rat arteries when subjected to stress or damage, but these lesions do not mimic atherosclerotic lesions since they are lipid poor, unless the rat's plasma cholesterol is artificially elevated either by a high cholesterol diet or injection of cholesterol rich lipoproteins (Bishop, 1980). Similarly, spontaneous arteriosclerotic lesions which appear in the rat do not stain for lipid and are more prevalent in the female. The occurance of these proliferative lesions was positively correlated to the frequency of breeding indicating there may be a hormonal influence to the proliferative disease (Wexler, 1964).

2. Diet

The rat is very resistant to dietary induction of atherosclerotic lesions (Bishop, 1980). Atherosclerotic lesions in rats similar to those seen in humans however, have been produced by feeding rats high cholesterol diets including bile acids and inducing hypothyroidism with

propylthiouracil or irradiation (Fillios et al., 1956; Malinow et al., 1954; Wissler et al., 1954). The rat's resistance to dietary induced atherosclerotic lesions is similar to that of the dog, which must also be made hypothyroid with a high cholesterol diet to induce atherosclerotic lesions (Mahley et al., 1977d). Another animal fairly resistant to dietary induction of atherosclerotic lesions is the mouse (Beher et al., 1963), whereas species that are susceptible to dietary induced atherosclerotic lesions include, rabbits, monkeys, swine and possibly humans (Camejo et al., 1973; Kramsch et al., 1981; Mahley, 1978; Ross, 1979; Shore et al., 1974).

3. Hypothyroidism

Hypothyroidism in the human has been found to cause a hyperlipidemia characterized by high plasma cholesterol levels, whereas thyrotoxicosis produces the opposite effect on plasma cholesterol and LDL levels (Mishkel et al., 1977; Walton et al., 1965). Thyroid hormones affect on cholesterol concentration of the plasma may be related to LDL receptors as it has been observed from in vitro studies on human skin fibroblasts that thyroxine stimulates the growth of LDL receptors (Chait et al., 1979). The increase in extrahepatic lipoprotein receptors would increase LDL catabolism in vivo and thus lower the plasma cholesterol concentration.

Experimentally induced hypothyroidism in rats has been found to affect the apo E and apo B composition of rat plasma lipoproteins. Moreover, both apo E and apo B concentrations increase by 100% after propylthiouracil induced hypothyroidism, with a concomitant increase in

plasma cholesterol of 50-100% and a 20-40% decrease in plasma triglycerides. The decrease in rat plasma triglycerides was believed to be due to an increase in lipoprotein lipase activity, whereas the increase in rat plasma cholesterol was due to a decrease in LDL and VLDL remnant removal (Dory et al., 1981). Hypothyroid rats on a high cholesterol diet produced qualitative and quantitative differences in the plasma lipoprotein profile as compared to euthyroid rats on the same high cholesterol diet. Plasma apo E concentrations decreased in the cholesterol fed euthyroid rats as compared to an increase in the hypothyroid cholesterol fed rats. In addition, HDL_c and beta migrating VLDL appeared in the hypothyroid cholesterol fed rat plasma, whereas neither lipoprotein was found in the plasma of euthyroid cholesterol fed rats. Apo B and plasma cholesterol were increased in both rat groups, but more so in the hypothyroid rats (DeLamatre et al., 1981). These data suggest that the hypercholesterolemia induced by hypothyroidism is qualitatively different from dietary induced hypercholesterolemia.

4. Miscellaneous Atherosclerotic Factors

HDL_c, the lipoprotein which appears after cholesterol feeding (see section II.D.3. Metabolism and Response to Hypercholesterolemia) may be involved with the atherosclerotic process, since HDL_c appears in all species studied when hypercholesterolemia has been induced (Mahley, 1978). On the other hand, the atherosclerotic process was shown to be inhibited in monkeys fed lanthanum with a high cholesterol atherogenic diet, therefore it was postulated that calcium, for which lanthanum is

an antagonist, is an integral part of the atherosclerotic process (Kramsch et al., 1981). Platelet derived growth factor (PDGF) has also been suggested as the initiator of the proliferative disorder associated with atherosclerotic lesions (Ross, 1979). Epinephrine administered with thyroxine and triiodothyronine was found to produce arteriosclerotic lesions in the rabbit, which was unrelated to any hypertensive effect (Oester, 1959). Currently, it would appear that no one event or factor may be responsible for the initiation and progression of the atherosclerotic lesion. The list of primary risk factors for atherosclerotic disease in humans includes, hyperlipidemia, hypertension, smoking and diabetes mellitus, with numerous possible secondary risk factors also implicated toward developing coronary artery disease (Grundy, 1983). Animal models of atherosclerotic disease have provided a great deal of information toward understanding the initiation and progression of atherosclerotic lesions. In addition, animal models have provide insights into lipoprotein metabolism and its relation to atherosclerotic disease. Animal models designed to investigate lipoprotein metabolism and atherosclerotic progression in response to the known and proposed risk factors can ultimately lead to the prevention of atherosclerotic disease.

CHAPTER II

RESEARCH OBJECTIVES

High density lipoprotein-cholesterol (HDL-C) has been implicated as a protective factor against atherosclerotic disease (Gordon et al., 1977; Miller et al., 1975). Since the rat is known to have a very large proportionate amount of HDL-C (Chapman, 1980; Frnka et al., 1974; Narayan, 1971) and is also relatively resistant to the experimental induction of atherosclerotic disease (Chapman, 1980; Fillios et al., 1956; Mahley et al., 1977a; Wissler et al., 1954), this animal would appear to be an excellent model to study the relationship of high density lipoproteins to atherosclerosis. Unfortunately, many studies which have utilized several different methods for determining plasma lipoprotein-cholesterol concentrations of the rat have yielded inconsistent results as indicated in a review by Chapman (1980). The coupling of an enzymatic determination of cholesterol (Allain et al., 1974) with cellulose acetate electrophoresis (Cobb et al., 1978) has made possible a rapid and specific separation and analysis of human plasma lipoprotein-cholesterol fractions, the ratios of which are currently being employed as risk factors for the development of human atherosclerosis. However, such a method has not been previously investigated with regard to the rat. The dissertation studies were therefore designed to compare the cellulose acetate electrophoretic plasma lipoprotein-cholesterol profile

of the rat and other experimental animals to that of the human. The presently reported studies were also designed to compare HDL-C levels in the rat determined by cellulose acetate electrophoresis to HDL-C levels obtained by polyanion precipitation techniques (Burstein et al., 1960; Kostner, 1976; Manual of Laboratory Operations, 1974). Therefore, the first part of this dissertation describes the technique by which we have characterized the cellulose acetate electrophoretic lipoprotein-cholesterol profile of the rat and its comparison to other experimental animals. These studies have led to the discovery of a lipoprotein unique to rat plasma and has been named by this laboratory for its electrophoretic mobility as prealbumin lipoprotein (PAL).

The second part of this dissertation deals with cholesterol movement between rat plasma lipoproteins and the subsequent discovery of a lipoprotein catabolic product possibly responsible for rat plasma cholesterol clearance. In addition, this dissertation also describes a new technique for determining the unesterified cholesterol (UC) concentration of plasma lipoprotein fractions separated by cellulose acetate electrophoresis. The presently described method for analyzing plasma lipoprotein-UC combined with the usual method for analyzing plasma lipoprotein-total cholesterol (TC) allows for the calculation of plasma lipoprotein-esterified cholesterol (EC) from the difference between plasma lipoprotein-TC and plasma lipoprotein-UC values. This new technique allowed us to characterize the in vitro movement of cholesterol between plasma lipoproteins more thoroughly. The transfer of cholesteryl ester between high density lipoprotein (HDL) and lower density

lipoproteins has previously been reported to occur in rat plasma at extremely low rates when compared to other species such as rabbits and humans (Barter et al., 1979c; Barter et al., 1978; Oschry et al., 1982). Recently however, two HDL subfractions that were isolated from rat plasma by HDL gradient ultracentrifugation were found to display cholesteryl ester transfer activity employing an in vitro assay. Rat plasma cholesteryl ester appeared to be transferred from a less dense HDL subfraction to a denser HDL subfraction, both isolated by density gradient ultracentrifugation (Jansen et al., 1983). In contrast, two rat plasma HDL subfractions isolated by zonal ultracentrifugation did not show any cholesteryl ester transfer activity in vitro. However in vivo, transfer of cholesteryl ester between these two HDL subfractions was observed with the transfer occurring from the denser HDL subfraction to the less dense HDL subfraction when the denser HDL subfraction containing radioactively-labeled cholesterol was administered to rats (Eisenberg et al., 1984). The conflicting observations of these two reports regarding cholesteryl ester transfer between various rat plasma HDL subfractions may be due to differences in lipoprotein compositions resulting from separation of lipoprotein fractions employing ultracentrifugal techniques (Lindgren et al., 1979). It has been suggested that electrophoretic techniques for isolation of plasma lipoprotein fractions may be superior to ultracentrifugal methods because of the distortions leading to lipoprotein degradation resulting from ultracentrifugation (Lindgren, 1980).

As determined from the first part of these dissertation studies, two of four rat plasma lipoproteins separated using the technique of

cellulose acetate electrophoresis combined with an enzymatic stain for total cholesterol (Cobb et al., 1978), were characterized as HDL subfractions and named according to their cellulose acetate electrophoretic mobilities as prealbumin lipoprotein (PAL) and alpha$_1$ lipoprotein. Side-by-side cellulose acetate electrophoretic comparisons between rat whole plasma and the rat plasma HDL fraction isolated in the density range between 1.063-1.210 g/ml by sequential ultracentrifugation (Havel et al., 1955), as well as the rat plasma HDL supernates obtained by manganese heparin precipitation (Manual of Laboratory Operations, 1974), and dextran sulfate precipitation (Kostner, 1976) confirmed that rat plasma prealbumin lipoprotein-cholesterol (PAL-C) and alpha$_1$ lipoprotein (α_1L-C) separated by cellulose acetate electrophoresis were HDL-C subfractions. The other two rat plasma lipoproteins isolated by cellulose acetate electrophoresis were alpha$_2$ lipoprotein-cholesterol (α_2L-C) corresponding to VLDL-C and beta lipoprotein-cholesterol (βL-C) corresponding to LDL-C. Studies presented in the second part of this dissertation were designed to investigate whether the two rat plasma high density lipoprotein subfractions, PAL and α_1L, were involved in the clearance of cholesterol from rat plasma, thereby playing a possible role in the rat's relative resistance to dietary-induced atherosclerotic disease (Chapman, 1980; Fillios et al., 1956; Mahley et al., 1977a; Wissler et al., 1954).

CHAPTER III

METHODS

I. SERUM COLLECTION

Samples of blood were obtained from the abdominal aorta of adult male Sprague-Dawley rats ranging from 60-90 days of age (Holtzman Co., Madison, WI) under light ether (Mallinckrodt, St. Louis, MO) anesthesia (Grice, 1964). Samples of rabbit blood were obtained from the ear vein of adult male New Zealand white rabbits (Langshaw Farms, Augusta, MI). Samples of mouse blood were obtained from neck vessels following decapitation of adult male Cox Swiss mice (Lab Supply Co., Indianapolis, IN). Samples of monkey blood were obtained from the femoral vein of phencyclidine-anesthetized adult male Rhesus monkeys (Primate Imports, New York, NY) courtesy of Dr. Randall from the department of Physiology. Samples of dog blood were obtained from the femoral artery of ethrane and nitrous oxide-anesthetized adult male dogs (Notsinger Kennels, St. Joseph, IL) courtesy of Dr. Thomas from the department of Physiology. Samples of human blood were obtained from the cubital vein of normal healthy adult male volunteers drawn while in a sitting position. All blood was collected in a sterile B-D vaccutainer tube with no additives (Becton, Dickenson and Co., Rutheford, NJ). The blood was placed in an ice bath, allowed to clot for 1 hour and then centrifuged at 1,000 x g for 15 min at 4 °C in an International refrigerated centrifuge. The

upper layer of serum was withdrawn and either placed in an ice bath for immediate analysis or frozen at -20 °C for analysis done at a later time.

II. SERUM AND PLASMA TOTAL CHOLESTEROL DETERMINATION

Serum or plasma total cholesterol levels were measured using an enzymatic method (Allain et al., 1974). Twenty microliters of serum or plasma were added to 1 ml of cholesterol reagent (Beckman Instruments Inc.) containing 1.6 mmol/l 4-aminoantipyrine, 21.2 mmol/l phenol, 50,000 IU/l peroxidase, 500 IU/l cholesterol oxidase and 440 IU/l cholesterol esterase in pH 7.5 phosphate buffer with cholate ions. Samples were mixed and incubated for 10 min at 37 °C. Absorbance was determined at 500 nm using a Beckman DU-8 spectrophotometer. Cholesterol concentrations were determined by comparison to a standard curve or by using a Beer Lambert constant. The assay was linear from 5 mg/dl to 500 mg/dl of cholesterol.

III. LIPOPROTEIN-CHOLESTEROL AND PROTEIN DETERMINATION

A. Lipoprotein-cholesterol Electrophoresis

Electrophoresis was carried out according to the method of Cobb and Sanders (1978) on Titan III cellulose acetate plates (Helena Laboratories, Beaumont, TX) that were soaked overnight for optimal resolution in pH 8.8, 0.077 ionic strength tris-barbital buffer (Helena Laboratories). Two microliters of serum or plasma were applied to the plate. The plate was electrophoresed at 180 volts for 25 min. Ten min prior to the end of the electrophoresis period, half of a second cellulose ace-

tate plate which had soaked for at least 15 min in 0.5 M, pH 6.7 phosphate buffer (Helena Laboratories) and blotted was then covered with enzymatic total cholesterol-staining reagent (Helena Laboratories) which was reconstituted with 1.5 ml of distilled water prior to electrophoresis. The electrophoresed sample plate was then cut in two, one half of which was layered carefully on top of the reagent plate and excess reagent and air bubbles removed, making a sandwich of the two half plates. The sandwiched plates were placed between two preheated development weights and incubated on top of a Helena Laboratories microevaporator hood for 25 min at 37 °C, following which the two plates were separated and the sample plate dried and examined under transmitted light for orange-colored lipoprotein-cholesterol bands. The orange-colored lipoprotein-cholesterol bands were densitometrically scanned at 500 nm in a Beckman DU-8 spectrophotometer with an attached densitometer. Lipoprotein-cholesterol concentrations were calculated by multiplying the relative percent of each lipoprotein fraction, as obtained by densitometric scanning, by the total serum or plasma cholesterol concentration.

B. Protein Staining

Half of the original sample plate containing duplicate samples of serum was stained for protein with Ponceau S (Briere et al., 1964). At the end of electrophoresis half of the sample plate was placed in 100 ml of Ponceau S protein stain (Helena Laboratories) for six minutes. The Ponceau S stained plate was then placed in 100 ml of 5% acetic acid three times for two minutes each with agitation. After the acetic acid

washes the Ponceau S stained plate was placed in 100 ml of absolute methanol (J. T. Baker Co., Phillipsburg, NJ) twice for two minutes each with agitation to dry the plate. Finally the Ponceau S stained plate was cleared in a solution consisting of 71 ml absolute methanol, 25 ml glacial acetic acid (DuPont de Nemoirs Co., Wilmington, DE) and 4 ml of Clear Aid (Helena Laboratories) for seven and a half minutes. The Ponceau S stained plate was then held vertically for one minute to remove excess clearing agent and then dried. The red-colored protein bands were densitometrically scanned at 525 nm in a Beckman DU-8 spectrophotometer with an attached densitometer.

C. "Total" Lipid Staining

"Total" lipid staining was also initially performed on electrophoresed serum samples as a comparison to the total cholesterol stained samples. The electrophoresed serum samples on the cellulose acetate plate were placed in a well containing 35 ml of oil red O stain (Helena Laboratories) to which 10 ml of one normal sodium hydroxide was added drop by drop with agitation five minutes prior to the end of electrophoresis. The electrophoresed cellulose acetate plate was soaked in the oil red O stain at room temperature for one hour, following which the stained plate was rinsed in water for a few seconds. The oil red O stained cellulose acetate plate was then wiped with cotton, placed in 100 ml of absolute methanol for 10 seconds, followed by 15 seconds in 100 ml of distilled water. After the water wash the plate was placed for 5-10 minutes in clearing solution consisting of 25 ml methanol and 100 ml of glycerol (Mallinckrodt). The oil red O stained plate was

removed from the clearing solution, blotted twice and five minutes later sprayed with Lipo Spray (Helena Laboratories) to fix the stain. The red-colored lipid bands were scanned densitometrically at 525 nm in a Beckman DU-8 spectrophotometer with an attached densitometer.

IV. ULTRACENTRIFUGAL ISOLATION OF RAT SERUM LIPOPROTEINS

Ultracentrifugal isolation of the rat serum HDL fraction was performed according to the method of Havel, Eder and Bragdon (1955). The serum of two rats each was pooled from 12 rats total, making six serum samples from which HDL was isolated. One tenth ml of 5% EDTA (Baker) was added to every 10 ml of rat serum used. A stock salt solution of density 1.346 g/ml was made using 15.3 g sodium chloride (Baker) and 35.4 g potassium bromide (Mallinckrodt) and making it up to 100 ml with distilled water. The density of the serum was adjusted to 1.063 g/ml by the following formula:

$$(A \times Y) + (B \times Z) = (A + B)X$$

where A is the volume of serum or serum infranate used, B is the volume of stock salt solution, Y is the nonprotein solvent density of serum or serum infranate, Z is the density of the stock salt solution and X is the desired nonprotein solvent density of the sample. The density adjusted serum was place in a 10.4 ml capacity, polycarbonate, ultracentrifuge bottle (Beckman Instruments Inc.) and centrifuged using a type 40 rotor in a Beckman Model L ultracentrifuge at 40,000 rpm for another 24 hours at 15 °C. The top two ml of supernate representing the density less than 1.063 g/ml fraction was removed and the remaining infranate

was washed in a density 1.063 g/ml salt solution and recentrifuged for another 24 hours. Following the removal of the density less than 1.063 g/ml fraction from the serum, the infranate was adjusted to a density of 1.210 g/ml by adding a concentrated salt solution of NaCl and KBr (density 1.346 g/ml) according to the formula as mentioned above. The sample was mixed and centrifuged using a type 40 rotor in a Beckman Model L ultracentrifuge at 40,000 rpm for another 24 hours at 15 °C. The top 1.0 ml of the supernate representing the HDL fraction (density 1.063-1.210 g/ml) was removed, washed with a density 1.210 g/ml salt solution and centrifuged at 40,000 rpm for 24 hours. Again the top 1 ml of the supernate was removed and the final washed 1.063-1.210 g/ml density HDL fraction was dialyzed using tubing (Spectrum Medical Ind. Inc., Los Angeles, CA) which was prewashed in distilled water for one hour. Dialysis was performed overnight at 4 °C against 15 liters of 0.15 M NaCl solution containing 0.05% EDTA and then electrophoresed on a cellulose acetate plate as described above. One experiment was performed in which the density 1.063 infranate was dialyzed as described above without adjusting the density to 1.210, therefore the infranate represented the density greater than 1.063 g/ml plasma fraction.

V. POLYANIONIC PRECIPITATION OF RAT SERUM LIPOPROTEINS

A. Manganese Heparin Precipitation

Manganese heparin precipitation of rat serum lower density lipoproteins was performed using a modification of the Lipid Research Clinics Program Method (Manual of Laboratory Operations, 1974). A 0.1 ml aliquot of HDL reagent containing 1,012 mmol/l manganese chloride and

2,000 U/ml heparin (Worthington Diagnostics, Freehold, NJ) was added to 1.0 ml of serum. The sample was vortexed 5 sec, placed in an ice bath for 30 min and then centrifuged at 1,000 x g for 30 min at 4 °C. The supernate containing the HDL-cholesterol was removed, analyzed for total cholesterol, dialyzed overnight at 4 °C against 0.15 M NaCl containing 0.05% EDTA and then electrophoresed on a cellulose acetate plate as described above.

B. Dextran Sulfate Precipitation

Dextran sulfate precipitation of rat serum lower density lipoproteins was performed according to the method of Kostner (1976). A 0.05 ml aliquot of dextran sulfate reagent containing 20 g/l dextran sulfate (Dow Diagnostics Co., Indianapolis, IN) was added to 1.0 ml of serum. Following mixing, 0.1 ml of magnesium sulfate reagent containing 1.1 mol/l magnesium sulfate (Dow Diagnostics Co.) was added and the mixture allowed to stand at room temperature for 5 min, after which it was centrifuged at 1,000 x g for 10 min at 4 °C. The supernate containing the HDL cholesterol was removed, analyzed for total cholesterol and then electrophoresed on a cellulose acetate plate as described above.

VI. RAT PLASMA COLLECTION

Male Sprague Dawley rats between the ages of 60-90 days obtained from Holtzman Co. (Madison, WI) were fed a diet of rat chow from Ralston Purina Co. (St. Louis, MO) ad libitum and had free access to water. The rats were on a light-dark cycle of 12 hours light from 0600-1800 and 12 hours dark from 1800-0600 for 2 weeks prior to the collection of blood.

Samples of blood were obtained from the abdominal aorta of rats under light ether anesthesia (Grice, 1964) employing vaccutainer tubes containing EDTA (Becton Dickenson, Rutheford, NJ). The tubes containing the blood were inverted several times and immediately placed in an ice bath, following which the blood was then centrifuged at 1500 x g for 30 minutes at 4 °C. The upper layer of plasma was collected and either placed in an ice bath for immediate analysis or frozen at -20 °C for analysis done at a later time.

VII. PLASMA INCUBATION

Each plasma sample was initially divided into three 1 ml portions: the first 1 ml portion of plasma was subdivided into 3 individual aliquots, each being placed in a 400 microliter stoppered micro test tube and immediately frozen at -20 °C for non-incubated t_0 samples so that there would only be a single thaw per sample prior to each analysis. The second 1 ml portion of plasma was placed in a small test tube containing 0.8 mg of p-chloromercuriphenylsulfonic acid (PCMPS), a lecithin:cholesterol acyltransferase (LCAT) inhibitor (Glomset et al., 1973) (Sigma Chemical Co., St. Louis, MO), while the third 1 ml portion of plasma was placed into an empty test tube. A 250 microliter aliquot of the second and third 1 ml portions of plasma was drawn into a siliconized Natelson blood collecting pipet, tightly sealed at both ends and then incubated at 37 °C for 4 hours. After the incubation period the pipettes were placed in an ice bath for 5 minutes and the incubated plasma then subdivided into 3 individual aliquots, each being placed in a 400 microliter stoppered micro test tube and immediately frozen at -20

°C for incubated t_4 hour samples.

VIII. PLASMA UNESTERIFIED CHOLESTEROL DETERMINATION

A. Enzymatic Determination of Unesterified Cholesterol

1. Quinoneimine Dye Generation

A method derived from the method developed by Allain and company (1974) was tested by this laboratory and compared with a commercial kit for the enzymatic determination of unesterified cholesterol and a gas chromatographic method for unesterified cholesterol determination. Unesterified cholesterol reagent was prepared fresh as needed and consisted of 5.0 mM sodium cholate (Sigma), 2.0 mM 4-aminoantipyrine (Sigma), 30,000 U/liter horseradish peroxidase type I (Sigma), 150 U/liter cholesterol oxidase (Sigma), 20 mM phenol (Mallinkrodt) and 0.17 mM polyethyleneglycol-8000 (Sigma), prepared in a 0.1 M, pH 6.7 phosphate buffer. Fifty microliters of plasma, standard or water (reagent blank) were added to test tubes containing one ml of the unesterified cholesterol reagent. The tubes were mixed and incubated at 37 °C for 20 minutes after which they were placed in a room temperature water bath for 5 minutes. Quinoneimine was generated in direct proportion to the amount of unesterified cholesterol present. Absorbance was determined at 500 nm using a Beckman DU-8 spectrophotometer.

2. Lutidine Dye Generation

Plasma unesterified cholesterol levels were measured by an enzymatic method (Beutler et al., 1976). Two hundred microliters of rat plasma were added to 2.5 ml of cholesterol color reagent (Boehringer Mannheim Co., Indianapolis, IN), mixed, then divided into two equal aliquots. One aliquot received 10 microliters of cholesterol oxidase (Boehringer Mannheim) and the other aliquot was used as a sample blank. The aliquots were covered, mixed and incubated at 37 °C for one hour. Lutidine dye was generated in direct proportion to the amount of unesterified cholesterol present. Absorbance was determined at 405 nm using a Beckman DU-8 spectrophotometer.

B. Gas Chromatographic Determination of Cholesterol

Both plasma unesterified and total cholesterol were determined by gas chromatography (Macgee et al., 1973). Twenty mg of 5 α-cholestane (Sigma), used as an internal standard, were dissolved in 1 ml diethylether and 20 ml of tetramethylammonium hydroxide (MCB, Cincinnati, OH) and then diluted to 100 ml with isopropanol (Mallinckrodt). This solution known as TMH-i was used for total cholesterol determination. For unesterified cholesterol determination, 10 mg of 5 α-cholestane were dissolved in 1 ml diethylether and diluted to 100 ml with isopropanol.

Two hundred microliters of TMH-i solution were added to 13x100 screw cap pyrex tubes. Two hundred microliters of the internal standard solution for the determination of unesterified cholesterol were added to separate tubes. Fifty microliters of plasma were added to each tube and mixed for 10 sec. The tube containing plasma and TMH-i solution was

placed in a heating block set at 70-80 °C for 15 min. The tube was then removed and 300 microliters of ethylacetate (Baker) added to the tube for the determination of unesterified cholesterol and the TMH-i tube. All tubes were mixed for 15-30 sec then centrifuged for 4 min at 500 x g at room temperature. One to two microliters of the clear upper phase were injected into a Varian 3740 gas chromatograph with a flame ionization detector containing a 4 ft. silanized glass column packed with 3% OV17 and HP chrom W 100/120 support (Varian, Co. Sunnyvale, CA). The injector temperature was 300 °C, the column temperature was 250 °C and the detector temperature was 350 °C. Unesterified and total cholesterol were both determined by calculating the peak height ratio of cholesterol to the internal standard. The resultant ratio was divided by the ratio obtained in a similar manner from a cholesterol standard solution which had been treated in the same manner as the plasma samples undergoing analysis. The cholesterol/internal standard peak height ratio determined from the plasma sample divided by the cholesterol/internal standard peak height ratio of the standard solution multiplied by the concentration of the standard solution equals the concentration of cholesterol in the plasma sample.

D. Esterified Cholesterol and LCAT Activity Determination

The plasma esterified cholesterol level was calculated as the difference between the plasma total and plasma unesterified cholesterol concentrations. Rat plasma LCAT activity was determined by the percent decrease in unesterified cholesterol concentration from the initial unesterified cholesterol concentration after a 4 hour 37 °C <u>in vitro</u>

incubation (Stokke et al., 1971; Patsch et al., 1976).

IX. LIPOPROTEIN-UNESTERIFIED AND ESTERIFIED CHOLESTEROL

A. Lipoprotein-unesterified Cholesterol Staining

Electrophoresis was carried out as previously described. Ten minutes prior to the end of the electrophoresis period, a cellulose acetate plate which had been soaked for at least 15 minutes in 0.5 M, pH 6.7 phosphate buffer, was removed from the buffer and blotted. The blotted plate was then covered with 1.5 ml of an enzymatic cholesterol-staining reagent similar to the total cholesterol-staining reagent, but lacking cholesterol esterase, therefore this reagent stained only the unesterified cholesterol of plasma lipoproteins separated by cellulose acetate electrophoresis. The enzymatic unesterified cholesterol reagent which was freshly prepared in our laboratory contained 1.0 U/ml cholesterol oxidase (Sigma), 100 U/ml horseradish peroxidase Type I (Sigma), 20.0 mM sodium cholate (Sigma), 4.0 mM 4-aminoantipyrine (Sigma) and 50.0 mM phenol (Mallinkrodt Inc. Paris, KY) in 0.5 M sodium phosphate buffer, pH 6.7. The electrophoresed plate was carefully layered on top of the reagent plate and excess reagent and air bubbles were removed making sandwiches of the plates. The sandwiched plates were then placed between two preheated development plates and incubated on top of a Helena Laboratories microevaporator hood for 25 minutes at 37 °C. After the incubation, the sample plate was separated from the reagent plate, dried and examined under transmitted light for orange-colored lipoprotein-unesterified cholesterol bands. The orange-colored lipoprotein-unesterified cholesterol bands were scanned at 500 nm employing a Beckman

DU-8 spectrophotometer with an attached densitometer.

B. Lipoprotein-Esterified Cholesterol

Plasma lipoprotein-total cholesterol electrophoresis and plasma lipoprotein-unesterified cholesterol electrophoresis combined with plasma total and unesterified cholesterol determinations result in mg/dl values for the total cholesterol (TC) content of each rat plasma lipoprotein fraction which included both unesterified cholesterol (UC) and esterified cholesterol (EC), as well as the unesterified (UC) content of each plasma lipoprotein fraction. In addition, the esterified cholesterol (EC) content of each plasma lipoprotein fraction was obtained from the difference between total cholesterol (UC + EC) and unesterified cholesterol (UC) values.

X. ESTROGEN INDUCED HYPOCHOLESTEROLEMIA

The effect of an increased plasma cholesterol clearance on rat plasma lipoprotein-cholesterol fractions separated by cellulose acetate electrophoresis was studied following the induction of lipoprotein receptor production by 17 α-ethinyl estradiol (Windler et al., 1980b). Adult male rats were administered either 17 α-ethinyl estradiol (Sigma) dissolved in propylene glycol (Sigma) or propylene glycol alone in daily subcutaneous doses for four days. All rats were then sacrificed on day 5. Two rats received 0.05 mg/kg of 17 α-ethinyl estradiol daily, four rats received 0.5 mg/kg of 17 α-ethinyl estradiol daily, four rats received 1.0 mg/kg of 17 α-ethinyl estradiol daily, and four rats received 2.5 mg/kg of 17 α-ethinyl estradiol daily. Five rats received

amounts of propylene glycol equivalent to that which was administered to the 17 α-ethinyl estradiol-treated rats. Rats were weighed daily and their food and water intake measured. Collection and incubation of plasma samples from adult male rats treated with 17 α-ethinyl estradiol was performed as previously mentioned.

XI. STATISTICS

A. Mean, Standard Deviation and Standard Error

The most commonly used parameter to describe the central character of data is the mean ($\bar{x}$) (Remington et al., 1970) which is the average value of all numbers (x) within an experimental population (n). To calculate the mean the resultant values of the data collected for the entire experimental population (n) are summed then divided by n:

$$\bar{x} = \sum_{i=1}^{n} x_i/n.$$

The standard deviation (s) is used to describe the dispersion of data around the mean in the same units as the mean. This statistic is derived from the measure of variability known as the variance. The variance is the average squared deviation of the measurements from their mean, while the standard deviation is the square root of the variance:

$$s = \left(\sum_{i=1}^{n} (x_i-\bar{x})^2/(n-1)\right)^{\frac{1}{2}}.$$

The standard error to the mean (S.E.M.) is calculated by dividing the standard deviation (s) by the square root of the experimental population (n):

$$S.E.M. = s/(n)^{\frac{1}{2}}.$$

Whereas the standard deviation is a useful statistic for describing the variability of the measurement, the standard error is used to establish a confidence interval for the true mean of a population and is useful for comparison of means to each other (Marks, 1982).

B. Linear Regression

Linear regression is used to determine the relationship of one variable to another. A correlation coefficient of 1, means the variables show a strong positive correlation, whereas a correlation coefficient of -1, means the variables show a strong negative correlation and a correlation of 0, means there is no correlation between the two variables. Linear regression analysis chooses as the best estimate a line for the data points by the least squares procedure (Remington et al., 1970). The algebraic equation for a line is:

$$y = mx + b$$

where m is the slope of the line and b is the intercept at the y axis. The slope of the line is estimated by summing the products of all variables x and y and subtracting from that the product of the sums of all x and the sums of all y divided by n. This result is divided by the square of sums of all x which is then divided by n and subtracted from the sum of all x squared:

$$m = \left(\left(\sum_{i=1}^{n} x_i y_i\right) - \left(\sum_{i=1}^{n} (x_i)\sum_{i=1}^{n} (y_i)\right)/n\right) / \left(\sum_{i=1}^{n} x_i^2\right) - \left(\sum_{i=1}^{n} x_i\right)^2/n.$$

The y intercept is estimated by the following formula:

$$b = (\sum_{i=1}^{n} yi - m\sum_{i=1}^{n} xi)/n.$$

Finally the correlation coefficient is described by :

$$r = m(s_x/s_y)$$

where s_x is the standard deviation of x and s_y is the standard deviation of y. The significance of r can be estimated by calculating its t statistic as follows:

$$t = (r^2(n-2)/(1-r^2))^{\frac{1}{2}}$$

The t value is compared to the values in a table of critical values for t with n-2 degrees of freedom for a two-tailed test. The coefficient of determination is r^2, which describes the amount of variation in the response variable measurement that can be explained by the fitted regression line. Therefore, a correlation coefficient of 0.5 yields a coefficient of determination of 0.25 meaning that the model explains 25% of the variability in the response variable measurements (Marks, 1982)

C. Paired Student's t-test

The paired t-test is used when two samples of data are obtained from the same subject (Remington et al., 1970). Plasma incubated four hours was compared to the fresh sample using the paired t-test. The t statistic was determined as follows:

$$t = \bar{d}/s_{\bar{d}}$$

where d is the mean difference between the paired observations and $s_{\bar{d}}$ is the standard error of the mean difference $\bar{d}$.

D. Analysis of Variance

Analysis of variance (ANOVA) was designed to compare more than two factor levels in a single test. This is necessary since multiple statistical testing decreases the overall significance level of the test (Marks, 1982). The increase in the overall significance level (OSL) can be determined from the following formula:

$$OSL = 1-(1-p)^k$$

where k is the number of independent tests and p is the level of significance. It can readily be seen that a statistical test used with a significance level (p) equalling 0.05 and only one comparison the OSL will remain at 0.05, but if three comparisons are made then the OSL increases to 0.14 and with four comparison to 0.26. Therefore, multiple testing increases the likelihood of rejecting the null hypothesis when it is true. The formula for one way ANOVA is as follows:

$$F=\left(\left(\sum_{i=1}^{n} T_i^2/n_i - T^2/n\right)/(k-1)\right)/\left(\left(\sum_{i=1}^{n}\sum_{i=1}^{n} x_{ij}^2 - \left(\left(\sum_{i=1}^{n} T_i^2/n_i\right)/(n-k)\right)\right)\right)$$

where k is the number of populations sampled, j is the index of the observation within samples, i is the index of sampled populations, n_i is the number of observations in sample i, n is the total number of observations, x_{ij} is the j^{th} observation in the i^{th} sample, T is the sum of all observations, T_i is the sum of the observations in sample i. The degrees of freedom (df) in the numerator is k - 1 and in the denominator

is n - k (Remington et al., 1970). The F statistic is calculated and compared to values in a table for the level of significance. If the level of significance is less than 0.05 then there is a significant difference between the means which were being compared, but the test will not determine which means were different.

E. Duncan's New Multiple Comparison Test

The ANOVA determined whether a difference existed between compared means and the Duncan's New Multiple Range Test was designed to determine which means were different with a constant overall significant level. Using the Duncan's test the difference between means must exceed a critical value to be considered significantly different. The critical value is determined using the mean squared error obtained from the analysis of variance, the number of samples (n) and the value q, which is obtained from a table. The value q is dependent on the mean squared error degrees of freedom (ν), the level of significance (α) and the number of steps (r) each mean is from the mean to which it is being compared. The formula for the critical difference (CD) is as follows:

$$CD = q_{\alpha;r;\nu} \, (MSE/n)^{\frac{1}{2}}.$$

The MSE is the denominator of the ANOVA equation with degrees of freedom of n-k (Kirk, 1982).

CHAPTER IV

RESULTS

I. COMPARISON OF RAT PLASMA LIPOPROTEIN AND PROTEIN PROFILES

Figure 3 presents a comparison of the cellulose acetate electrophoretic migration of adult male rat serum proteins stained for total cholesterol and for protein. Four orange-colored cholesterol-staining lipoprotein bands were detected in the serum of adult male rats and have been named according to their plasma protein-staining electrophoretic mobilities. Beta lipoprotein-cholesterol, abbreviated as βL-C, had β1-globulin mobility corresponding to low density lipoprotein (LDL) and accounted for an average of 13.6% of the total plasma lipoprotein-cholesterol. Alpha$_2$ lipoprotein-cholesterol, abbreviated as α_2L-C, had α_2-globulin mobility corresponding to very low density lipoprotein (VLDL) and accounted for 5.1% of the total plasma lipoprotein-cholesterol. Alpha$_1$ lipoprotein-cholesterol, abbreviated as α_1L-C had α_1-globulin mobility corresponding to high density lipoprotein (HDL) and accounted for 55.9% of the total plasma lipoprotein-cholesterol. A fourth band was found to migrate faster than rat albumin with prealbumin mobility accounting for the remaining 25.4% of the total plasma lipoprotein-cholesterol, and has been presently named as prealbumin lipoprotein-cholesterol and abbreviated as PAL-C.

Figure 4 compares a "total" lipid stain (oil red O) to the spe-

cific enzymatic cholesterol stain both performed after cellulose acetate electrophoresis. The cholesterol stain offers sharper resolution between the lipoprotein bands without staining albumin as appeared to occur with the "total" lipid stain. Moreover, quantitation of lipoprotein-cholesterol could be performed using the enzymatic cholesterol stain, whereas the "total" lipid stain was semiquantitative at best. However, the "total" lipid stain did offer the advantage of staining lipoproteins containing high concentrations of triglycerides such as chylomicrons, which usually have a very low cholesterol content and could not be seen by a specific cholesterol stain.

Overnight-fasting of adult male rats did not affect the relative percentages of the presently obtained lipoprotein-cholesterol profile. However, the resolution of PAL-C was markedly influenced by the amount of time the cellulose acetate plate was presoaked in tris-barbital buffer as shown in figure 5. It was found that overnight soaking of the cellulose acetate plate was necessary for the optimal resolution of PAL-C from α_1L-C. Although it is not clear as to why the resolution was improved by increased soaking time, it is possible that the cellulose acetate plate's absorptivity changes upon prolonged soaking which could improve the resolution between PAL-C and α_1L-C bands found in rat plasma.

II. SPECIES COMPARISON

Figure 6 presents the comparative distribution of plasma lipoprotein-cholesterol fractions separated by cellulose acetate electrophoresis in normal adult male rat, mouse, rabbit, dog, monkey and human serum samples. PAL-C found in rat serum was not present in serum samples of the adult mouse, rabbit, dog, monkey or human, indicating that PAL may be a new plasma lipoprotein with a cellulose acetate electrophoretic mobility faster than albumin. The present data also suggest that PAL may be a plasma lipoprotein that may be unique to the rat. All the species studied except the human were found to have higher amounts of α_1L-C compared to their amounts of βL-C present.

Serum lipoprotein-cholesterol values in terms of mg/dl of the various normal adult nonfasting animals studied by cellulose acetate electrophoresis are given in table 3. The concentration of PAL-C and α_1L-C in adult male rat serum was found to be 15 ± 4 (± S.D.) and 33 ± 7 mg/dl, respectively. The sum of PAL-C and α_1L-C of the adult male rat was found to be 81.4% of the total serum cholesterol concentration which appears to agree with previous reports in the literature for the total amount of rat serum high density lipoprotein-cholesterol as determined by ultracentrifugal separation of lipoproteins (Frnka et al., 1974; Havel et al., 1955; Manninen et al., 1978; Narayan et al., 1968; Narayan, 1971). One of the currently-used coronary artery disease risk ratios obtained by dividing the LDL-cholesterol (LDL-C) concentration by the HDL-cholesterol (HDL-C) concentration (Gordon et al., 1977) was found to be lowest for those species least responsive to diet-induced

atherogenesis (rat, mouse and dog) and highest in those species (rabbit, monkey and human) most prone to diet-induced atherogenesis (Beher et al., 1963; Camejo et al., 1973; Kramsch et al., 1981; Mahley et al., 1974; Mahley, 1978; Ross, 1979; Shore et al., 1974). In addition, the adult male human was also found to differ markedly from the experimental animals studied with regard to another currently-employed coronary artery disease risk ratio (Gordon et al., 1977), having a serum total cholesterol to HDL-cholesterol ratio almost three times as great as the other species.

III. PAL: A HIGH DENSITY LIPOPROTEIN

Figure 7 characterizes PAL-C and α_1L-C separated by cellulose acetate electrophoresis as rat plasma high density lipoprotein-cholesterol employing ultracentrifugation (Havel et al., 1955). Only two cholesterol-staining bands were found to occur in the high density fraction(1.063-1.210 g/ml) of normal adult rat serum separated by ultracentrifugation. These two bands had cellulose acetate electrophoretic mobilities corresponding to PAL-C and α_1L-C, indicating that both belong to the HDL-cholesterol fraction of rat plasma. Moreover, figure 8 demonstrates essentially the same comparison of the denser plasma fraction (greater than 1.063 g/ml) to whole serum indicating that PAL and α_1L are both high density lipoproteins. However, the dialyzed plasma fraction with a density greater than 1.063 g/ml demonstrates better resolution between the PAL and α_1L bands than the rat plasma fraction separated between the densities 1.063-1.210 g/ml. This could be due to the stabilizing influence of albumin, which improved the electrophoretic resolu-

tion of plasma proteins when paper was used as a support medium (Lees et al., 1963). Confirmation that PAL and α_1L were high density lipoproteins of the adult rat was achieved by using two different polyanionic precipitation methods (Kostner, 1976; Manual of Laboratory Operations, 1974). The manganese heparin supernate containing the high density lipoprotein fraction of rat plasma was dialyzed prior to cellulose acetate electrophoresis in order to remove the manganese, since manganese interferes with the enzymatic cholesterol reagent (Steele et al., 1976; Thompson et al., 1981). Dialysis of the dextran sulfate supernatant fraction containing the high density lipoprotein fraction of rat plasma prior to cellulose acetate electrophoresis was found to be unnecessary. Two cholesterol-staining bands were also found to occur in both the 1,000 x g manganese heparin high density lipoprotein supernate and the 1,000 x g dextran sulfate high density lipoprotein supernate, with electrophoretic mobilities corresponding to PAL-C and α_1L-C, as shown in figures 9 and 10. These results offer additional evidence that both PAL-C and α_1L-C belong to the HDL-C fraction of rat plasma. It is also interesting to note that albumin is present in the HDL supernates of both precipitation methods and the resolution of PAL from α_1L is equivalent to that of the density greater than 1.063 g/ml plasma fraction indicating that albumin may be a factor in the increased resolution of PAL from α_1L using cellulose acetate electrophoresis.

IV. METHOD COMPARISON

Figures 11 and 12 show that a positive correlation using linear regression analysis exists between rat serum HDL-C levels determined by cellulose acetate electrophoresis and rat serum HDL-C levels determined by either the manganese heparin precipitation method or by the dextran sulfate precipitation method. The total HDL-C concentration for the cellulose acetate electrophoretic method was obtained by adding the PAL-C and α_1L-C concentrations. Figure 13 demonstrates that the best correlation exists between the two precipitation techniques as would be expected. Although the three methods demonstrated a statistically significant positive correlation, figure 14 shows that statistically significant differences in absolute HDL-C levels in identical samples of adult male rat serum were obtained by each method. Statistical comparisons were made using an analysis of variance and Duncan's new multiple range test. Mean values for adult male rat HDL-C concentration obtained by cellulose acetate electrophoresis were 44 ± 2 mg/dl (± S.E.M.), with the mean PAL-C being 12 ± 1 mg/dl and the mean α_1L-C being 32 ± 2 mg/dl. In contrast, mean values for the HDL-C concentration obtained by manganese heparin precipitation and dextran sulfate precipitation were 34 ± 2 and 27 ± 2 mg/dl respectively. Not only did both polyanionic precipitation methods yield statistically significant different values from those obtained by the electrophoretic method, but both polyanionic precipitation methods yielded statistically significant different values from each other.

V. RAT PLASMA LIPOPROTEIN-CHOLESTEROL MOVEMENT

Figure 15 shows a progressive decrease in α_1L-TC concentration along with a progressive increase in PAL-TC concentration during a 4 hour incubation of adult rat plasma at 37 °C. At the end of the 4 hour incubation period, PAL-TC had increased from 25.0% to 63.2% of the plasma lipoprotein-cholesterol while α1L-TC had decreased from 51.9% to 21.4% of plasma lipoprotein-cholesterol. No statistically significant change in plasma total cholesterol was observed during the 4 hour *in vitro* incubation period. These data indicated a marked movement of total cholesterol from α_1L to PAL during the *in vitro* incubation period. Figure 16 depicts the changes in the lipoprotein profile as demonstrated densitometrically both at 1 hour and 4 hours after incubation at 37 °C. Although not clearly seen in figure 16, table 4 demonstrates that incubation of rat plasma appears to affect the electrophoretic mobility of βL and to some extent the mobility of α_2L as βL moves toward the α_2L region. βL increases its mobility by 25.8% whereas α_2L electrophoretic mobility increased by 11.9%. PAL and α_1L mobility were unaffected by the incubation. Interestingly, the addition of 2.0 mM PCMPS a known LCAT inhibitor (Glomset *et al.*, 1970) increased the mobility of βL slightly, but not significantly, while after a 4 hour 37 °C *in vitro* incubation βL mobility does not differ significantly from the initial mobility as compared to the fresh sample containing 2.0 mM PCMPS. However, βL electrophoretic mobility increased significantly in the sample containing PCMPS when compared to the fresh sample with no additives. It is possible that LCAT may be responsible for the increase in electrophoretic mobil-

ity of βL, but PCMPS, which is a relatively nonspecific sulfhydryl group inhibitor (Vansteveninck et al., 1965) could have affected other plasma enzymes or the LDL molecule specifically, preventing the increased mobility that was seen after a four hour in vitro incubation of rat plasma. The observed increase in βL electrophoretic mobility and lipoprotein-cholesterol movement between lipoproteins was an indication that a dynamic process continued to exist in plasma incubated in vitro at physiological temperatures and was an indication of possible problems that could occur if a plasma sample was not analyzed immediately after being taken or steps are not taken to inhibit either lipoprotein-cholesterol movement or increased lipoprotein electronegativity. Moreover, it was an indication that a process could be occurring in vivo affecting rat plasma lipoprotein-cholesterol composition and lipoprotein integrity, which we attempted to study by in vitro incubations.

Figure 17 indicates that statistically significant changes occurred in plasma lipoprotein-UC concentrations in 3 out of 4 rat plasma lipoprotein fractions during a 4 hour 37 °C in vitro incubation of plasma. The decrease in α_1L-TC concentration appears to be due to a change in α_1L-UC which decreased by 202 ± 5 nmol/ml plasma/4 hr since α_1L-EC remained unchanged. The increase in PAL-TC concentration which amounted to 295 ± 18 nmol/ml plasma/4 hr appears to be due to both an increase in PAL-UC of 75 ± 16 nmol/ml plasma/4 hr and an increase in PAL-EC of 220 ± 21 nmol/ml plasma/4 hr. In addition, βL-UC was found to decrease by 41 ± 10 nmol/ml plasma over the 4 hour incubation period, while βL-EC remained unchanged. The observed loss of UC from βL and α_1L

together with the concomitant increase in PAL-EC suggested that either the UC of βL was first transferred to α_1L, where the UC was esterified by LCAT to EC which was then transferred to PAL or on the other hand, the UC of βL and α_1L was transferred directly to PAL, where the UC was then esterified. These two possibilities were therefore investigated by inhibiting the esterification of rat plasma by the addition of 2.0 mM PCMPS, a known LCAT inhibitor (Glomset et al., 1970).

VI. LIPOPROTEIN-CHOLESTEROL MOVEMENT WITH LCAT INHIBITION

Confirmation that rat plasma UC esterification is inhibited by the addition of PCMPS was obtained by the following observations. In the absence of PCMPS, rat plasma UC was found to decrease by 38.4 ± 4.4% following a 4 hour 37 °C in vitro incubation of plasma. In the presence of 2.0 mM PCMPS however, rat plasma UC was found to decrease by only 3.3 ± 0.8% under identical conditions. Figure 18 shows that in the presence of 2.0 mM PCMPS, instead of a loss of UC from α_1L occurring in incubated rat plasma, there was a 44 ± 13 nmol/ml plasma/4 hr increase in α_1L-UC, along with a loss of 41 ± 5 nmol/ml plasma/4 hr in the βL fraction, demonstrating that rat plasma βL-UC concentrations decreased by the same amount during a 4 hour incubation period of plasma both in the presence or absence of 2.0 mM PCMPS. These results indicated that UC was transferred from βL to α_1L since the amount of α_1L-UC increase was equal to the βL-UC decrease over the 4 hour incubation period in LCAT-inhibited plasma. In addition, these data also suggested that following the transfer of UC from βL to α_1L in rat plasma incubated without 2.0 mM PCMPS, the transferred UC was then esterified, and the resulting

EC either transferred to PAL or alternatively, α_1L itself might have been partially catabolized to PAL, causing the presently observed increased concentration of PAL-EC.

A cholesteryl ester transfer factor may be present in rat plasma since PAL-EC decreased by 59 ± 34 nmol/ml plasma/4 hr while α_1L-EC increased by 93 ± 39 nmol/ml plasma/4 hr in adult rat LCAT inhibited plasma following a 4 hour 37 °C *in vitro* incubation. This suggested that cholesteryl ester transfer may occur between the two rat plasma HDL subfractions from PAL to α_1L, but does not appear to be physiologically significant since it was only observed in LCAT inhibited plasma. However, it may be significant that the increase of 14% in rat plasma α_1L-EC in the presence of 2.0 mM PCMPS observed in the present studies was similar to the increases in VLDL cholesteryl ester found in rat plasma incubated under similar conditions as previously reported using ultracentrifugation to separate lipoproteins in order to assay for lipoprotein-cholesteryl ester transfer activity (Barter *et al.*, 1978). These data indicate that rat plasma may possess a lipoprotein-cholesteryl ester transfer factor inhibitor as has previously been reported to be present in the rat and other species lacking significant lipoprotein-cholesteryl ester transfer activity (Morton *et al.*, 1981).

The α_2L fraction was the only lipoprotein fraction of rat plasma whose cholesterol concentration did not appear to be affected by the 4 hour 37 °C *in vitro* incubation. The α_2L-TC concentration did not significantly change nor did the α_2L-EC or α_2L-UC concentration in adult male rat plasma incubated either in the absence or presence of 2.0 mM

PCMPS, indicating that LCAT had no effect on α_2L-cholesterol concentration.

Figures 19 and 20 present changes in esterified to unesterified cholesterol ratios of lipoprotein fractions separated by cellulose acetate electrophoresis following a 4 hour 37 °C in vitro incubation of adult male rat plasma in the presence and absence of 2.0 mM PCMPS. The EC/UC ratio of α1L increased by 500% in plasma incubated in the absence of PCMPS because of a decrease in UC without any change in EC as shown in figure 17. The EC/UC ratio of βL showed a statistically significant increase after incubation of rat plasma both in the presence and absence of 2.0 mM PCMPS because of a decrease in βL-UC without any change in the βL-EC concentration. α_2L appeared to have a relatively constant EC/UC ratio throughout the in vitro incubation. The observed increase in the EC/UC ratio of α_1L incubated rat plasma is most likely due to LCAT esterification of α_1L-UC. However, inasmuch as the α_1L-EC concentration was found to be unchanged during the incubation period, this suggests that the EC generated by LCAT activity must in some way have been incorporated into PAL, which was the only lipoprotein fraction in which a significant increase in EC concentration occurred when rat plasma was incubated without PCMPS.

It is possible that the incorporation of EC from α_1L into PAL may be due to a specific transfer of EC from α_1L to PAL, but this would not explain the subsequent increase in UC necessary to keep the EC/UC ratio of PAL constant during the incubation period. In addition, the EC transfer from α_1L to PAL, was not demonstrated in LCAT-inhibited plasma,

although LCAT inhibition by PCMPS does not appear to affect the transfer of cholesteryl esters between plasma lipoproteins of other species (Ha et al., 1982). Another explanation of our data may be that PAL is the preferred substrate for rat plasma LCAT and that UC from both α_1L and βL is passively transferred to PAL, where it is then esterified by LCAT. That βL may be passively transferred is suggested since there is no evidence for an energy requiring process or transfer protein in order that unesterified cholesterol may be moved from one lipoprotein to another. However, when PCMPS was added to rat plasma causing an inhibition of LCAT, a transfer of UC did occur from βL to α_1L. It has been previously reported that equilibration of UC between plasma lipoproteins is slow relative to LCAT activity. If LCAT is inhibited, then the preferred LCAT substrate may be relatively unsaturated with respect to UC, and in order to regain equilibrium, UC would have to be transferred to the preferred LCAT substrate to achieve relative saturation and equilibrium with the other plasma lipoproteins (Perret et al., 1983). It would therefore be expected that if PAL were the preferred rat LCAT substrate, then UC should have been transferred from βL to PAL to establish equilibrium once LCAT is inhibited. The data however, suggests that α_1L and not PAL is the preferred substrate for rat LCAT activity, since α_1L receives βL-UC as presently demonstrated in LCAT-inhibited rat plasma. Still another explanation of our data is that in the rat, PAL is a catabolic product of α_1L, the α_1L in some way splitting to form a PAL particle containing a specific amount of UC and EC. Further investigation of this possibility was therefore performed using the technique of obtain-

ing rats with hypocholesterolemia that is induced by the *in vivo* administration of 17 α-ethinyl estradiol (Davis *et al.*, 1978).

VII. RAT PLASMA LIPOPROTEIN-CHOLESTEROL CLEARANCE

Adult male rats administered subcutaneous doses of 17 α-ethinyl estradiol dissolved in propylene glycol did not differ significantly from rats receiving only propylene glycol with regard to body weight or food intake. Figure 21 shows that the plasma total cholesterol concentrations of rats receiving 17 α-ethinyl estradiol were markedly lower than vehicle control rats. The plasma total cholesterol of adult male rats administered 0.05 mg/kg of 17 α-ethinyl estradiol daily was significantly lower after 5 days than the control group by an average of 50.9%. Rats receiving daily doses of 0.5 mg/kg of 17 α-ethinyl estradiol had 73.6% lower plasma cholesterol levels after 5 days than control rats. Rats receiving daily doses of 1.0 mg/kg of 17 α-ethinyl estradiol had 90.6% lower plasma cholesterol than control rats and those rats receiving daily doses of 2.5 mg/kg of 17 α-ethinyl estradiol had 81.1% lower plasma cholesterol values at 5 days compared to control rats.

Figure 22 presents the effects of *in vivo* administration of 17 α-ethinyl estradiol on lipoprotein-total cholesterol concentrations before and after a 4 hour 37 °C *in vitro* incubation of adult male rat plasma. The lower plasma cholesterol values observed after 17 α-ethinyl estradiol administration are primarily reflected in the values of PAL-TC and βL-TC. The lowest dose of 17 α-ethinyl estradiol (0.05 mg/kg daily for 4 days) resulted in PAL-TC levels that were 96.7% lower than control rats and βL-TC levels that were 92.9% lower than control rats. Both

PAL-TC and βL-TC apparently disappeared from the plasma of rats receiving 0.5 mg/kg and higher doses of 17 α-ethinyl estradiol, whereas α_1L-TC and α_2L-TC remained present at all doses of 17 α-ethinyl estradiol even though both α_1L-TC and α_2L-TC showed decreased levels. The cholesterol present in the plasma of adult male rats administered 0.5 mg/kg and higher of 17 α-ethinyl estradiol therefore appeared to be carried exclusively by α_1L and α_2L. Only PAL-TC reappeared following a 4 hour in vitro incubation of those plasma samples in which PAL-TC was previously not detected as a result of an in vivo 17 α-ethinyl estradiol administration. In addition, a concomitant decrease in α_1L-TC was found to occur in incubated plasma of rats administered 0.5 mg/kg and higher 17 α-ethinyl estradiol. Unlike PAL-TC, βL-TC could not be detected in incubated plasma of rats administered 0.5 mg/kg and higher 17 α-ethinyl estradiol either before or after a 4 hour 37 °C in vitro incubation. The fact that PAL-TC reappeared in the plasma of 17 α-ethinyl estradiol-treated rats after a 4 hour in vitro incubation at 37 °C and that α_1L-TC decreased during the same incubation with LCAT remaining active suggests that PAL of the rat may be a product of plasma α_1L catabolism and that α_1L is the preferred substrate for rat LCAT activity, as summarized in figure 23. In addition, α_1L-cholesterol appears to be linked to LCAT activity since PAL-cholesterol concentrations did not change as drastically when rat plasma was incubated at 37 °C in the presence of PCMPS, an LCAT inhibitor, which is summarized in figure 24. However, statistically significant changes in the cholesterol concentration of α_1L, PAL and βL still occur. These changes appear to reflect the processes of

unesterified and possibly esterified cholesterol transfer. PAL-TC decreased due to a decrease in PAL-EC while α_1L-TC increased as a result of both an increase in UC and EC. In addition βL-TC decreased as a result of a UC decrease. The increase in α_1L-UC was the same as the βL-UC decrease indicating that the UC most probably came to α_1L from βL. The EC drop in PAL with a concomitant increase in α_1L-EC indicated that transfer of EC may have occurred from PAL to α_1L under these conditions. Moreover, the present data suggest that PAL, like βL, may be cleared by hepatic lipoprotein receptors of the rat since both PAL-TC and βL-TC appear to be rapidly removed from rat plasma following the induction of lipoprotein receptor production by 17 α-ethinyl estradiol.

CHAPTER V

DISCUSSION

I. CELLULOSE ACETATE LIPOPROTEIN-CHOLESTEROL ELECTROPHORESIS

Resolution of plasma lipoproteins by paper electrophoresis has been greatly improved by employing a barbital-albumin buffer (Lees et al., 1963). However, paper electrophoresis of plasma lipoproteins generally requires 16 hours for completion. Cellulose acetate used as a support medium for electrophoresis is capable of excellent resolution of plasma lipoproteins without the requirement of albumin in the buffer, within 15-45 minutes (Beckering et al., 1970; Chin et al., 1968; Fletcher et al., 1970). Accurate quantitation of electrophoretically-separated plasma lipoproteins with nonspecific "total" lipid stains has been difficult, because of each lipoprotein's differing affinities for such stains (Fletcher et al., 1970). Such commonly used lipid stains as Sudan black B, preferentially stain unsaturated sterol esters and unsaturated triglycerides, whereas unesterified cholesterol and phospholipids are stained negligibly or not at all (Schjeide et al., 1963). Moreover, the various cholesteryl esters and triglycerides have different staining affinities for Sudan black B, dependent upon their particular fatty acids. Oil red O, which is another commonly used "total" lipid stain, stains cholesteryl esters more intensely than Sudan black B, but its staining is also dependent on the cholesterol ester's degree

of fatty acid saturation. In addition, oil red O also stains triglycerides as well as albumin, but does not stain phospholipids or unesterified cholesterol. Recently a cellulose acetate electrophoretic technique, which combines an enzymatic staining procedure for total cholesterol (Allain et al., 1974) has been developed for determining human plasma lipoprotein-cholesterol concentrations which are currently employed as risk factors for human atherosclerotic disease (Cobb et al., 1978). The method of quantitating human plasma lipoproteins using enzymes specific for cholesterol is more precise and accurate as opposed to "total" lipid staining.

Ultracentrifugation and polyanionic precipitation techniques, although commonly used for human plasma lipoprotein fractionation, may not be ideal methods for the analysis of rat plasma lipoprotein-cholesterol concentrations. Ultracentrifugation may lead to lipoprotein degradation and loss of lipoprotein-cholesterol in the rat (Fainaru et al., 1977; Marcel et al., 1981) as well as the loss of apolipoproteins from HDL in the human (Hojnacki et al., 1978; Lindgren et al., 1979). Another drawback to ultracentrifugation is the time involved in separating lipoprotein fractions, which can take up to a week to accomplish (Havel et al., 1955). A major problem with precipitation methods is that although the various lipoprotein density classes can be sequentially precipitated, the resolution between lipoprotein classes is so close that a portion of the lipoprotein class which is desired in solution may also occur in the precipitate (Burstein et al., 1970). In a study comparing various precipitation techniques to sequential ultracen-

trifugation (Warnick et al., 1979a; 1979b), a good correlation was found between the methods however, differing HDL-cholesterol levels were obtained depending on which method was used. This would indicate that the various precipitation techniques are not equivalent and can lead to significant differences in HDL-C quantitation. Another problem with precipitation techniques is the inadvertent precipitation of HDL containing apolipoprotein E along with the apolipoprotein B-containing lipoproteins (Mahley et al., 1977b).

Cellulose acetate electrophoresis coupled with an enzymatic cholesterol stain offers several advantages over other methods for measuring the distribution of rat plasma lipoprotein-cholesterol. The cellulose acetate electrophoretic method utilizes only 2 µl of serum which permits multiple sampling from the same animal. In contrast, both the polyanion precipitation and ultracentrifugal methods require several ml of serum. The cellulose acetate electrophoretic method has been presently found to fractionate rat plasma into four lipoprotein-containing cholesterol bands, compared to only three lipoprotein bands which have been observed in rat serum employing paper electrophoresis (Mahley et al., 1977a; Watson, 1961). Polyanion precipitation methods yield only two fractions, namely HDL-C in the supernate and lower density lipoprotein-cholesterol consisting of both VLDL-C and LDL-C as a precipitate. In addition, the present data indicates that polyanion precipitation methods employing rat serum resulted in a loss of 29-40% of the HDL-total cholesterol.

This dissertation describes a new method for the determination of

unesterified cholesterol in plasma lipoprotein fractions. The reagent we used was derived from the enzymatic technique for the measurement of plasma total cholesterol (Allain et al., 1974) and the method was derived from that for lipoprotein-total cholesterol analysis by cellulose acetate electrophoresis (Cobb et al., 1978). The unesterified cholesterol reagent was used to determine the UC concentrations of rat plasma and compared favorably to two established techniques for the determination of plasma UC (Beutler et al., 1976; Macgee et al., 1973) with identical results observed for all methods employed. We have used this method to describe the plasma cholesterol metabolism of the rat in an in vitro assay. This method has great potential for the study of cholesterol metabolism not only in the rat but other experimental systems as well including the human. The advantages of electrophoretic separation and specific cholesterol staining techniques include, small sample requirments, high resolution, short assay time, greater accuracy and precision and fewer artifacts as compared to other methods for plasma lipoprotein fractionation, such as ultracentrifugation or polyanionic precipitation (Fainaru et al., 1977; Hojnacki et al., 1978; Kunitake et al., 1982; Lindgren et al., 1979; Mahley et al., 1977b; Marcel et al., 1981; Warnick et al., 1979a; 1979b).

II. COMPARISON OF SPECIES PLASMA LIPOPROTEIN-CHOLESTEROL

Plasma lipoprotein-cholesterol ratios for various animal species have been obtained employing cellulose acetate electrophoretic separation of lipoproteins coupled with an enzymatic cholesterol stain. The LDL-C/HDL-C ratios were presently found to be markedly higher in the

rabbit, monkey and human, species known to be more prone to experimentally-induced atherosclerotic lesions (Camejo et al., 1973; Kramsch et al., 1981; Mahley, 1978; Ross, 1979; Shore et al., 1974), than the LDL-C/HDL-C ratios of the rat, mouse and dog, which are species less prone to induced atherosclerotic lesions (Beher et al., 1963; Fillios et al., 1956; Mahley et al., 1974; 1977b; Mahley, 1978; Wissler et al., 1954). The total cholesterol to HDL-C ratio was also found to be higher in those species most prone to induced atherosclerotic lesions, although the species differences between the total cholesterol/HDL-C ratios appear to be less marked than the species differences between the LDL-C/HDL-C ratios. Human epidemological evidence has shown that the LDL-C/HDL-C ratio is positively correlated to the risk of developing atherosclerotic disease (Gordon et al., 1977), therefore it is possible that a high LDL-C/HDL-C ratio may be an important indicator of species susceptibility to the experimental-induction of atherosclerotic disease.

III. PAL: A UNIQUE RAT PLASMA LIPOPROTEIN

The present data employing cellulose acetate electrophoresis combined with an enzymatic cholesterol stain clearly demonstrates for the first time that adult male rat plasma contains a rapidly-moving cholesterol-containing high density lipoprotein with an electrophoretic mobility equivalent to prealbumin. Previous electrophoretic studies of rat plasma apparently have not demonstrated prealbumin lipoprotein due to either differences in the electrophoretic medium or due to the semiquantitative nature of the "total" lipid stains employed (Fletcher et al., 1970; Schjeide et al., 1963). Agarose gel electrophoresis of rat plasma

HDL following ultracentrifugal separation demonstrated a single lipid-staining band whose migration appeared to include the prealbumin region (Koga et al., 1969). Agarose gel electrophoresis of rat whole plasma combined with a Sudan black B stain also appeared to suggest a prealbumin lipoprotein band, however in this system lipid-stained bands migrated consistently farther than their corresponding amido black protein-stained bands (Johansson et al., 1976). Human plasma alpha$_1$ lipoprotein also separated by agarose gel electrophoresis appeared to have the same electrophoretic mobility as albumin, with the lack of electrophoretic resolution between albumin and α_1L possibly being due to non-specific albumin staining (Johansson et al., 1976; Schjeide et al., 1963). In addition, agarose-starch gel electrophoresis of rat plasma using a combined oil red O and fat red 7B "total" lipid stain also appeared to suggest bands which had prealbumin, alpha$_1$, prebeta and beta electrophoretic mobility (Chalvardjian, 1971). The prealbumin and alpha$_1$ bands appeared to correspond to HDL, since after ultracentrifugal fractionation of the plasma, these two bands had a density range greater than 1.063 g/ml, while the prebeta band corresponded to VLDL, and the beta band corresponded to LDL. However, no attempt was made to determine the actual cholesterol content of the prealbumin-migrating lipoprotein band visualized by a "total" lipid stain. Moreover, a side-by-side electrophoretic comparison of total lipid-stained and protein-stained plasma was not performed using the agarose-starch gel electrophoretic technique and moreover, the prealbumin lipid-staining band was visualized from albumin by the difference in albumin's staining color. The

agarose-starch gel method combined with a "total" lipid stain is quite semiquantitative with densitometric analysis being extremely difficult due to albumin interference. On the other hand, the present studies employing cellulose acetate electrophoresis combined with an enzymatic cholesterol stain allow for the easy distinction between prealbumin lipoprotein and albumin, as well as an accurate comparative quantitation of the cholesterol concentration of prealbumin lipoprotein and other rat plasma lipoproteins.

Based on the smaller cholesterol content of PAL, as indicated in table 3, this high density lipoprotein fraction of rat plasma obtained by cellulose acetate electrophoresis may be similar to the HDL_1 subfraction isolated from rat plasma by rate zonal ultracentrifugation (Eisenberg et al., 1984) or by density gradient centrifugation (Jansen et al., 1983), which would therefore make $\alpha_1 L$ obtained by cellulose acetate electrophoresis similar to HDL_2 isolated by centrifugation. One problem with this interpretation is the rapid electrophoretic mobility of PAL on cellulose acetate, since apolipoprotein E-containing lipoproteins have been found to migrate in the α_2-globulin region using paper electrophoresis, and HDL_1 of the rat has been reported to have a high apolipoprotein E-content (Mahley et al., 1977a; Weisgraber et al., 1977). However, rat plasma PAL isolated by cellulose acetate electrophoresis may contain other apolipoproteins besides apolipoprotein E, which might enhance its electrophoretic mobility.

IV. FAST BETA LIPOPROTEIN

The lipid rich β fraction of human plasma was shown to be sensitive to storage and temperature since β globulin electrophoretic mobility increased when stored for long periods (Armstrong et al., 1947). A similar study demonstrated that within 36 hours human plasma β globulin acquires α_2 globulin electrophoretic mobility. However, the increase in mobility can be prevented for at least 10 days by storage at 0-4 °C or by heating the serum at 50 °C for 3 hours (Hoch et al., 1954), which would indicate that a plasma enzyme was probably involved in this electrophoretic transformation. More evidence of enzymatic involvement was indicated when the increase in βL electrophoretic mobility was inhibited by sodium bromoacetate or heating at 58 °C as demonstrated using paper electrophoresis with a "total" lipid stain after a 72 hour 37 °C incubation of either horse or human serum (Tayeau et al., 1956). We have redemonstrated this increase in beta lipoprotein electrophoretic mobility for the first time in rat plasma and have shown that PCMPS a known sulfhydryl group inhibitor (Velick, 1953) also inhibits the increased electrophoretic mobility of βL after a 37 °C in vitro incubation.

The increased mobility of β lipoprotein at first may have seemed trivial, an artifact of incubation, but in light of recent findings as to the increased uptake of electronegative LDL by the scavenger pathway (Brown et al., 1980), this change in mobility may have some influence on the pathogenesis of atherosclerosis. Human plasma β lipoprotein was shown to have increased electrophoretic mobility after it passed into the lymph (Reichl et al., 1973; 1975). Moreover, LDL incubated with

cultured endothelial cells was found to be more electronegative and had enhanced uptake by incubated macrophages (Henriksen et al., 1981). In addition, in vivo administration of the modified LDL particles resulted in an increased uptake into liver endothelial cells as opposed to the unmodified LDL which was taken up by liver parenchymal cells (Nagelkerke et al., 1984). Therefore, it has been demonstrated that increased electronegativity of LDL can occur in vivo as well as in vitro and that a pathway for the clearance of this more electronegative LDL exists. Moreover, the macrophage scavenger pathway, responsible for the clearance of LDL with increased electronegativity has been implicated as a factor leading to atherosclerotic lesions (Goldstein et al., 1979).

The mechanism for the increased mobility of LDL is not known though several possibilities may be explored. Heparin, an endogenously produced substance has been shown to increase the electrophoretic mobility of lipoproteins possibly by its activation of lipoprotein lipase (Houtsmuller, 1966) or by complexing with the LDL molecule as dextran sulfate complexes with LDL, thus increasing LDL's negative charge and its affinity for the macrophage receptor (Basu et al., 1979). The possibility exists that an enzyme may be involved which is heat labile and sensitive to sulfhydryl group inhibition. The enzyme could act in some way to degrade the LDL apolipoproteins, which could reveal more electronegative groups. Another possibility could be that the enzyme degrades the lipid content of LDL or transfers it to another lipoprotein, causing a conformational change which could reveal electronegative groups on the LDL molecule. One other possibility is the transfer of apolipoproteins

from other lipoproteins to the LDL molecule which could increase its electronegativity. These possibilities all remain to be explored in the future.

V. RAT PLASMA LIPOPROTEIN-CHOLESTEROL MOVEMENT

Esterified cholesterol transfer between HDL and VLDL particles isolated by ultracentrifugal techniques has been found to occur during the *in vitro* incubation of plasma of rabbits, humans and other animal species (Barter *et al.*, 1978; 1979; Ha *et al.*, 1982; Nichols *et al.*, 1965; Zilversmit *et al.*, 1975). However, esterified cholesterol transfer between plasma HDL and VLDL in rats has either not been observed (Oschry *et al.*, 1982) or has been reported to occur at such a low rate as to be physiologically insignificant (Barter *et al.*, 1978). The present studies suggest that movement of cholesteryl ester does occur from α_1L to PAL, two high density lipoprotein fractions of rat plasma separated by cellulose acetate electrophoresis. In contrast, the present data also suggest that when rat plasma LCAT was inhibited, lipoprotein-cholesteryl ester transfer activity may still be present, but EC movement was found to occur from PAL to α_1L rather than from a high density lipoprotein to the very low density lipoproteins as found in other species (Ha *et al.*, 1982). The observed cholesteryl ester movement from α_1L to PAL appears to be the physiologically significant process since movement of EC in the opposite direction could only be demonstrated when LCAT was inhibited. The presence in rat plasma of an inhibitor to cholesteryl ester transfer as has been previously reported (Morton *et al.*, 1981) would indicate that the significant movement of cholesteryl ester

from α_1L to PAL as presently reported was most probably due to LCAT activity and not to lipoprotein-cholesteryl ester transfer.

Figure 25 shows our proposed *in vivo* mechanism for cholesterol clearance by circulating plasma lipoproteins in the adult male rat based on current knowledge of rat lipoprotein metabolism and from the present data obtained from *in vitro* incubation studies of rat plasma coupled with 17 α-ethinyl estradiol-induced rat hypocholesterolemia. It would appear that α_1L is the preferred substrated for LCAT activity in rat plasma similar to HDL_3 being the preferred substrate for LCAT in humans (Fielding *et al.*, 1971; 1972a; Jahani *et al.*, 1981). The catabolic conversion of α_2L (VLDL) to βL (LDL) by the action of lipoprotein lipase (Eisenberg *et al.*, 1973a) occurs only *in vivo* since the enzyme is bound to the endothelial walls of the vasculature (Eisenberg *et al.*, 1982) and generally released by highly anionic compounds like heparin (Krauss *et al.*, 1973) and would therefore not be a factor in the present *in vitro* assay.

It has been reported that the uptake of cellular UC by HDL is determined by the rate of desorption of cholesterol out of the cell membrane into the aqueous phase surrounding the cell as well as the distance of diffusion to HDL (Phillips *et al.*, 1980). The rate of cholesterol efflux from cells may also be modulated by apolipoprotein A-I (Fielding *et al.*, 1981b). The transfer of UC to HDL is probably due to LCAT activity creating an unsaturated HDL particle with respect to UC, allowing more UC to accumulate on the HDL particle (Perret *et al.*, 1983). The exchange of UC between lipoproteins occurs constantly

(Roheim et al., 1963), but seems to be temperature dependent, since at 4 °C no exchange of UC occurs between lipoproteins (Perret et al., 1983). Net transfer of UC between plasma lipoproteins has been reported not to occur when LCAT is inhibited (Perret et al., 1983; Fielding et al., 1981a; 1981b), however, a net transfer of UC between βL and α_1L was observed during the presently reported in vitro incubation of rat plasma. A possible explanation for this net transfer of UC between rat βL and α_1L may involve α_1L being relatively unsaturated with respect to UC due to LCAT activity, with net transfer of UC from βL to α_1L occurring in order to achieve equilibrium. Moreover, the UC transfer from βL to α_1L observed in LCAT inhibited plasma incubated for 4 hours may reflect the same process indicating that esterification of UC by LCAT occurs at a faster rate than UC can be transferred to α_1L to achieve relative saturation, which would explain the 2-4 hours needed for equilbrium to be achieved between the UC concentrations of incubated rat plasma lipoproteins as has been reported previously (Perret et al., 1983; Roheim et al., 1963).

VI. RAT PLASMA LIPOPROTEIN-CHOLESTEROL CLEARANCE

17 α-Ethinyl estradiol has been reported to induce the production of hepatic lipoprotein receptors that are specific for apolipoprotein B (apo B) and apolipoprotein E (apo E) containing lipoproteins (Windler et al., 1980b), suggesting that PAL because of its rapid clearance from the plasma in 17 α-ethinyl estradiol-treated rats may therefore contain either of these two apolipoproteins. However, since PAL is a high density lipoprotein, it is more probable that PAL contains apo E rather

than apo B (Quarfordt et al., 1978). The fact that apo E-containing lipoproteins have been found to migrate to the α_2-globulin region using paper electrophoresis (Mahley et al., 1977a; Weisgraber et al., 1977) together with the finding that PAL migrates much further when electrophoresed on cellulose acetate, suggests that PAL may contain other apolipoproteins besides apo E which enhance its electrophoretic mobility. Apolipoprotein analysis of the cellulose acetate electrophoretically-separated rat plasma lipoproteins are currently being undertaken in this laboratory to determine which apolipoproteins are associated with PAL and α_1L in order to establish whether PAL and α_1L are similar to the ultracentrifugally-separated less dense HDL_1 and more dense HDL_2 subfractions of rat plasma. The conflicting reports of rat plasma cholesteryl ester transfer between HDL_1 and HDL_2 (Eisenberg et al., 1984; Jansen et al., 1983) or between HDL and VLDL (Barter et al., 1978) may be due to the apo E content of the lipoproteins since the apo E content has been found to be greatly affected by ultracentrifugal isolation of lipoproteins (Fainaru et al., 1977; Kunitake et al., 1982).

The present data suggest that the clearance of rat plasma cholesterol appears to be through an HDL pathway in which α_1L is catabolized to PAL in the plasma, with the catabolism of α_1L being apparently related to LCAT activity. PAL may then be removed from the plasma by hepatic lipoprotein receptors, which can be induced by 17 α-ethinyl estradiol (Windler et al., 1980). This is in marked contrast to the human in which cholesterol accumulates in LDL, as a result of cholesteryl ester transfer from HDL to VLDL (Barter et al., 1979b) with subse-

quent metabolism of VLDL to LDL (Eisenberg et al., 1973a). LDL is then cleared from human plasma by LDL receptors (Brown et al., 1981). Since HDL has been negatively correlated to the risk of developing atherosclerosis in humans (Miller et al., 1975), it is possible that the rat's relative resistance to dietary-induced atherosclerotic lesions (Fillios et al., 1956; Mahley et al., 1977a; Wissler et al., 1954) may be due to a major role that the HDL subfractions, α_1L and PAL, may play in clearing cholesterol from rat plasma.

CHAPTER VI

SUMMARY

The plasma lipoprotein-cholesterol profile of the adult male rat was investigated using cellulose acetate electrophoresis combined with an enzymatic stain for total cholesterol and an enzymatic stain for unesterified cholesterol developed for these studies. A comparison of the electrophoretically determined serum lipoprotein-cholesterol profile of the adult male rat with mouse, rabbit, dog, monkey and human serum was performed. Four cholesterol-staining lipoprotein bands were detected in rat serum, while only three bands were present in the other species studied. The apparently unique lipoprotein-cholesterol band in the rat was found to electrophoretically migrate faster than rat albumin with prealbumin mobility and has been named prealbumin lipoprotein-cholesterol (PAL-C). PAL-C of the rat, accounting for 25.4% of the total serum cholesterol of the adult male rat, was shown to be an HDL fraction employing ultracentrifugation as well as a polyanion precipitation technique. Therefore, HDL of rat plasma is composed of both a prealbumin and an α_1 lipoprotein fraction as can be determined by cellulose acetate electrophoresis.

It was found using cellulose acetate electrophoresis and staining for both total and unesterified cholesterol that following a four hour 37 °C in vitro incubation of rat plasma, the total cholesterol concen-

trations of both PAL and α_1L change due to a decrease in the unesterified cholesterol concentration of α_1L with a concomitant increase in the esterified cholesterol concentration of PAL. In the presence of PCMPS, a lecithin:cholesterol acyl transferase inhibitor, incubated rat plasma demonstrated a decrease in PAL-total cholesterol with a concomitant increase in α_1L-total cholesterol. In addition, a net transfer of unesterified cholesterol occurred from βL to α_1L, both in the presence and absence of PCMPS. Hypocholesterolemia induced by the *in vivo* administration of 17 α-ethinyl estradiol to rats resulted in the disappearance of both PAL and βL-total cholesterol from plasma. However, PAL-total cholesterol reappeared in rat plasma during an *in vitro* 4 hour incubation at 37 °C while α_1L-total cholesterol decreased. The present data suggest that both PAL-cholesterol and βL-cholesterol are cleared from rat plasma by hepatic lipoprotein receptors induced by 17 α-ethinyl estradiol and that PAL of the rat may be a product of plasma α_1L catabolism.

CHAPTER VII

FIGURES

Figure 1. Cholesterol

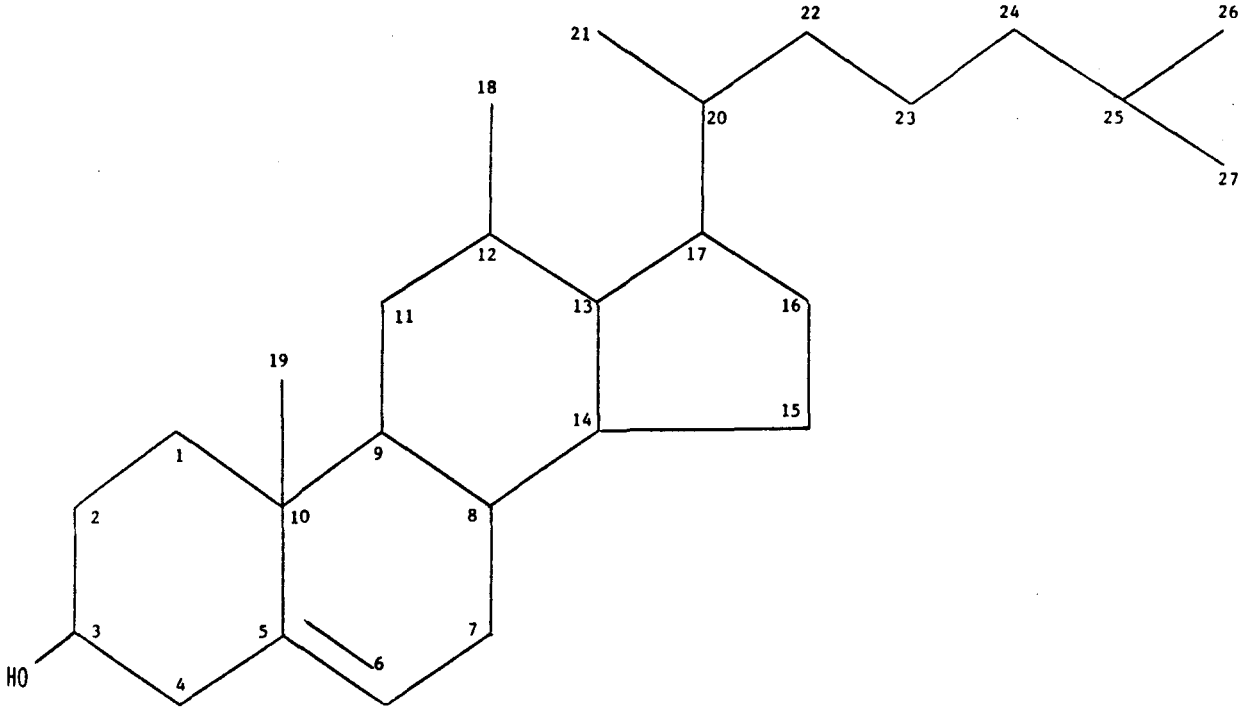
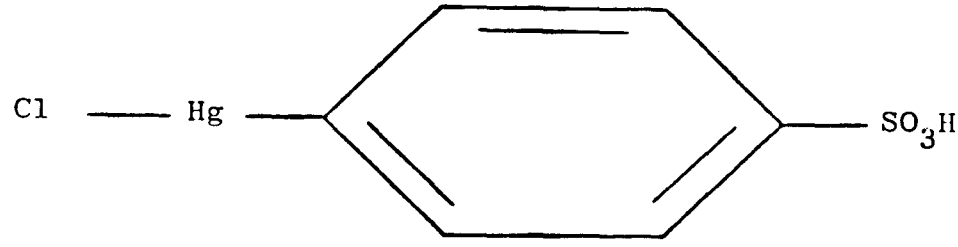


Figure 1

Figure 2a. p-Chloromercuriphenylsulfonic Acid

2b. p-Chloromercuriphenylsulfonate Sodium Salt (PCMPS)

a.



b.

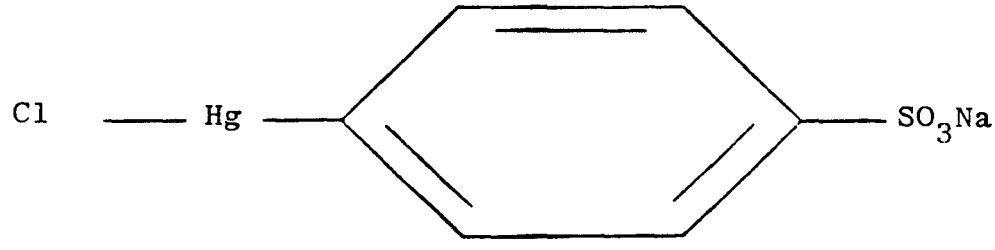


Figure 2

Figure 3. A comparison between the lipoprotein-cholesterol profile and the protein profile of rat serum as separated by cellulose acetate electrophoresis. Following electrophoresis of duplicate 2 microliter aliquots of serum on different halves of the cellulose acetate plate, the plate was cut into two equal parts. One part was enzymatically stained for total cholesterol and the second part stained for protein with Ponceau S. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol; PA is prealbumin; A is albumin; α_1 is alpha$_1$ globulin; α_2 is alpha$_2$ globulin; β_1 is beta$_1$ globulin; β_2 is beta$_2$ globulin; γ is gamma globulin.)

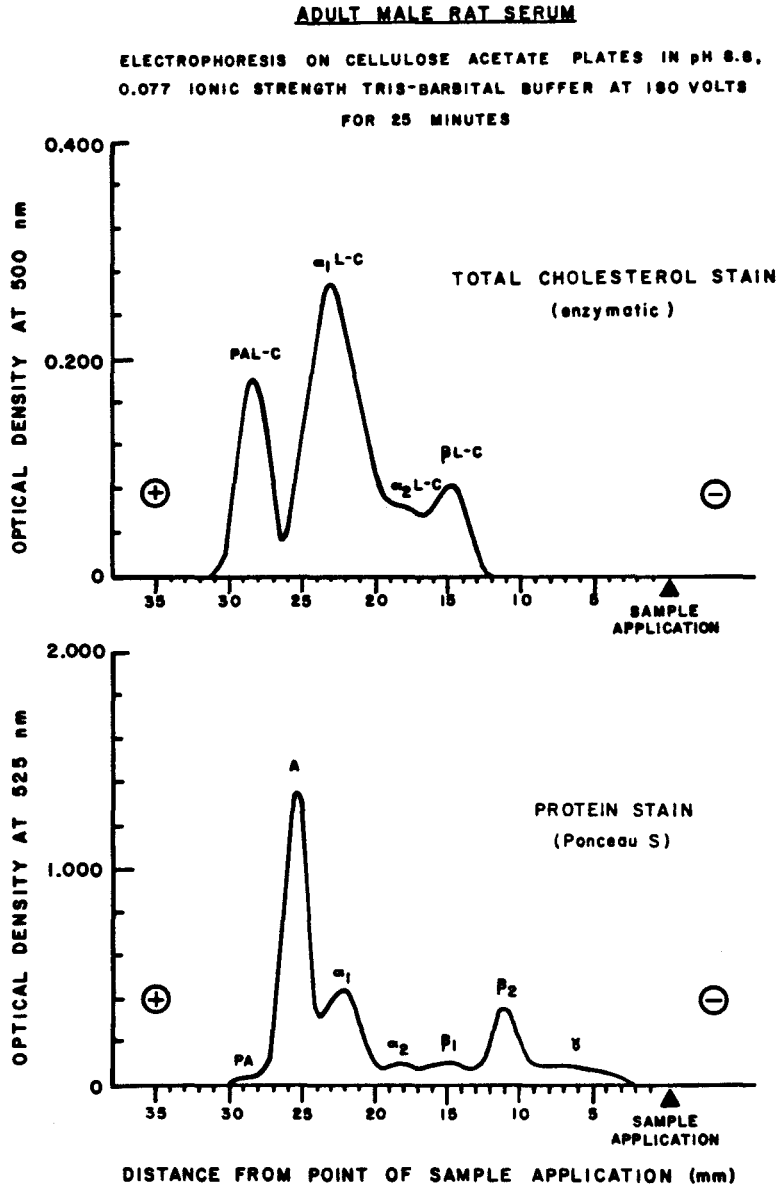

ADULT MALE RAT SERUM
ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.8, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER AT 180 VOLTS FOR 25 MINUTES
OPTICAL DENSITY AT 500 nm
0.400
0.200
0
TOTAL CHOLESTEROL STAIN
(enzymatic)
PAL-C
α1L-C
βL-C
α2L-C
35 30 25 20 15 10 5
SAMPLE APPLICATION
OPTICAL DENSITY AT 525 nm
2.000
1.000
0
PROTEIN STAIN
(Ponceau S)
A
PA
α1
α2
β1
β2
γ
35 30 25 20 15 10 5
SAMPLE APPLICATION
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 3

Figure 4. A comparison between the lipoprotein-cholesterol profile and the lipoprotein profile of rat serum as separated by cellulose acetate electrophoresis. Following electrophoresis of duplicate 2 microliter aliquots of serum on different halves of the cellulose acetate plate, the plate was cut into two equal parts. One part was enzymatically stained for total cholesterol and the second part stained for "total" lipids with oil red O. (PAL-C is prealbumin lipoprotein-cholesterol; HDL-C is high density lipoprotein-cholesterol; VLDL-C is very low density lipoprotein-cholesterol; LDL-C is low density lipoprotein-cholesterol; PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; pre-βL is prebeta lipoprotein; βL is beta lipoprotein.)

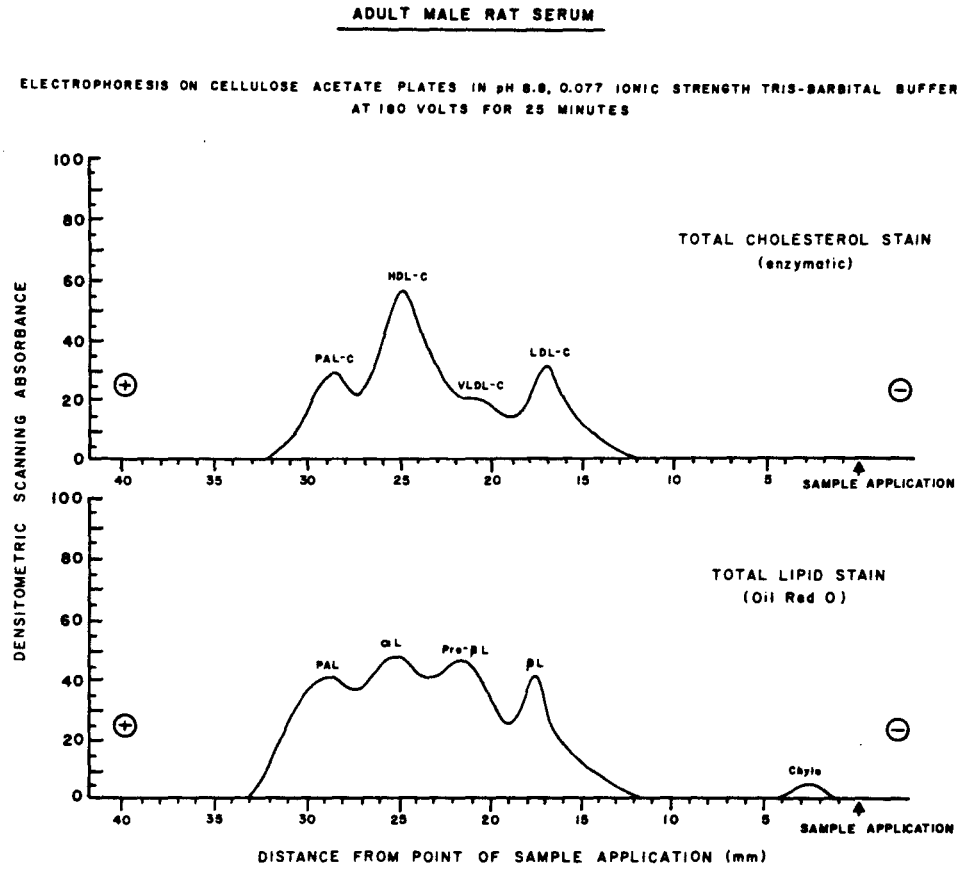

ADULT MALE RAT SERUM
ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.8, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER AT 180 VOLTS FOR 25 MINUTES
DENSITOMETRIC SCANNING ABSORBANCE
TOTAL CHOLESTEROL STAIN
(enzymatic)
PAL-C
HDL-C
VLDL-C
LDL-C
SAMPLE APPLICATION
TOTAL LIPID STAIN
(Oil Red O)
PAL
α L
Pre-β L
β L
Chyle
SAMPLE APPLICATION
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 4

Figure 5. Improved resolution of PAL-C from α_1L-C by soaking cellulose acetate plate overnight. Two microliter aliquots of adult rat plasma were applied to two cellulose acetate plates, one of which had been presoaked overnight in tris-barbital buffer; the other being presoaked for 1 hour in tris-barbital buffer. Both plates were then electrophoresed in the same chamber simultaneously. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol.)

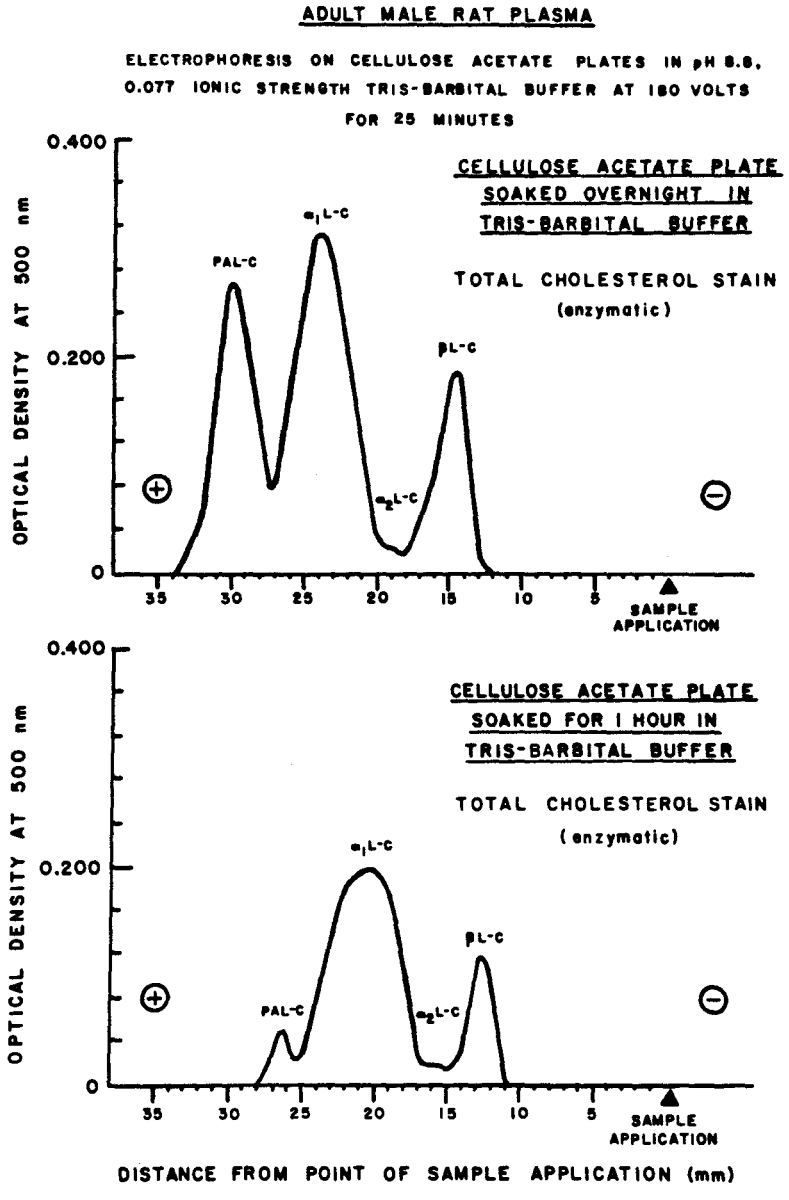

ADULT MALE RAT PLASMA
ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.8, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER AT 180 VOLTS FOR 25 MINUTES
OPTICAL DENSITY AT 500 nm
0.400
0.200
0
CELLULOSE ACETATE PLATE SOAKED OVERNIGHT IN TRIS-BARBITAL BUFFER
TOTAL CHOLESTEROL STAIN (enzymatic)
PAL-C
α1L-C
α2L-C
βL-C
35 30 25 20 15 10 5
SAMPLE APPLICATION
CELLULOSE ACETATE PLATE SOAKED FOR 1 HOUR IN TRIS-BARBITAL BUFFER
TOTAL CHOLESTEROL STAIN (enzymatic)
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 5

Figure 6. Comparison of the serum lipoprotein-cholesterol profiles of the normal adult male rat, mouse, rabbit, dog, monkey and human, determined by cellulose acetate electrophoresis combined with an enzymatic cholesterol stain. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol; PA is prealbumin; A is albumin; α_1 is alpha$_1$ globulin; α_2 is alpha$_2$ globulin; β_1 is beta$_1$ globulin; β_2 is beta$_2$ globulin; γ is gamma globulin.)

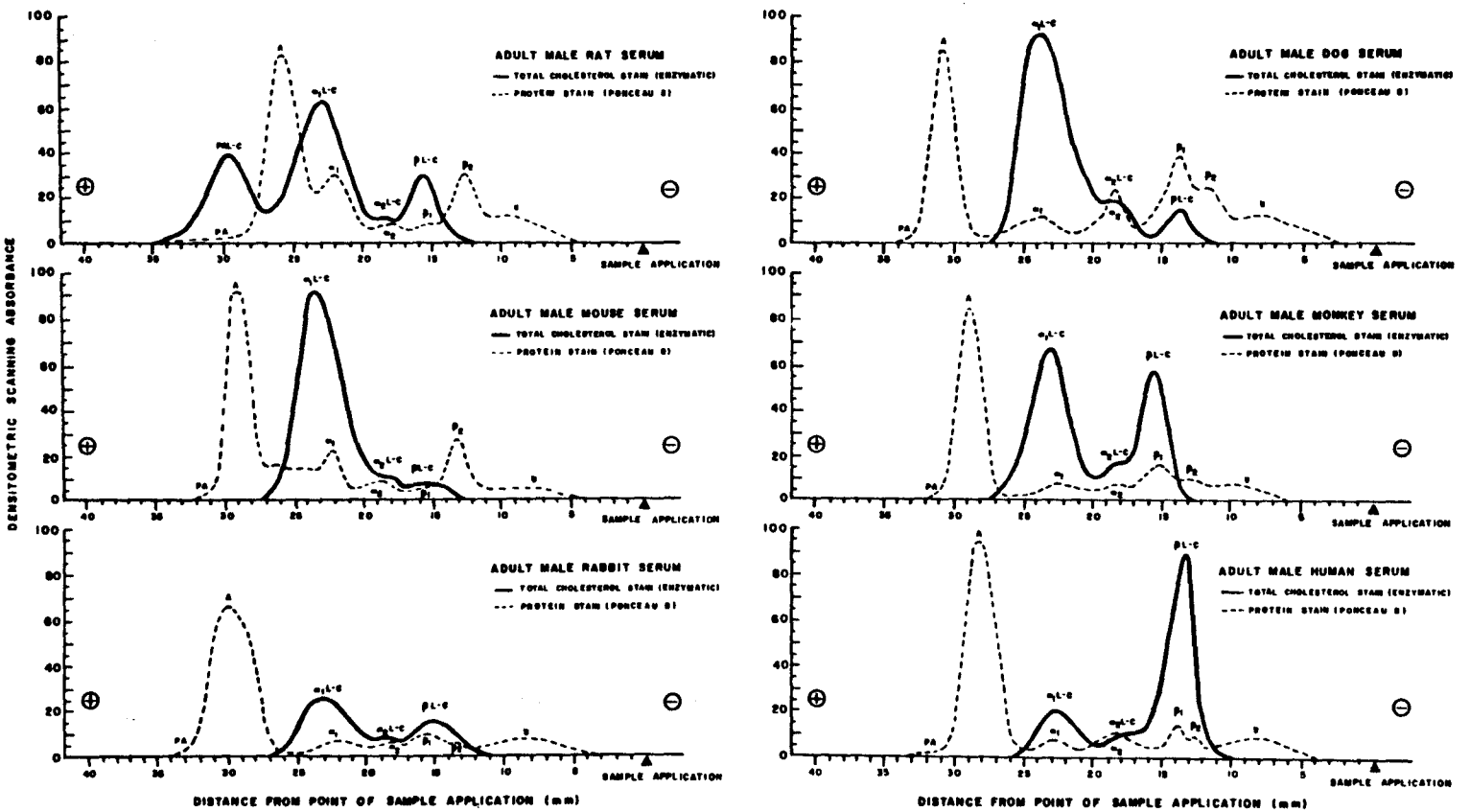

ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.6, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER
AT 180 VOLTS FOR 25 MINUTES
ADULT MALE RAT SERUM
ADULT MALE DOG SERUM
ADULT MALE MOUSE SERUM
ADULT MALE MONKEY SERUM
ADULT MALE RABBIT SERUM
ADULT MALE HUMAN SERUM
TOTAL CHOLESTEROL STAIN (ENZYMATIC)
PROTEIN STAIN (PONCEAU S)
SAMPLE APPLICATION
DENSITOMETRIC SCANNING ABSORBANCE
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 6

Figure 7. Cellulose acetate electrophoresis of the high density fraction (1.063-1.210 g/ml) of adult male rat serum obtained by ultracentrifugation. A sample of the corresponding whole serum was stored at -20 °C during the time required to isolate the high density fraction and was subsequently electrophoresed on the same plate. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol.)

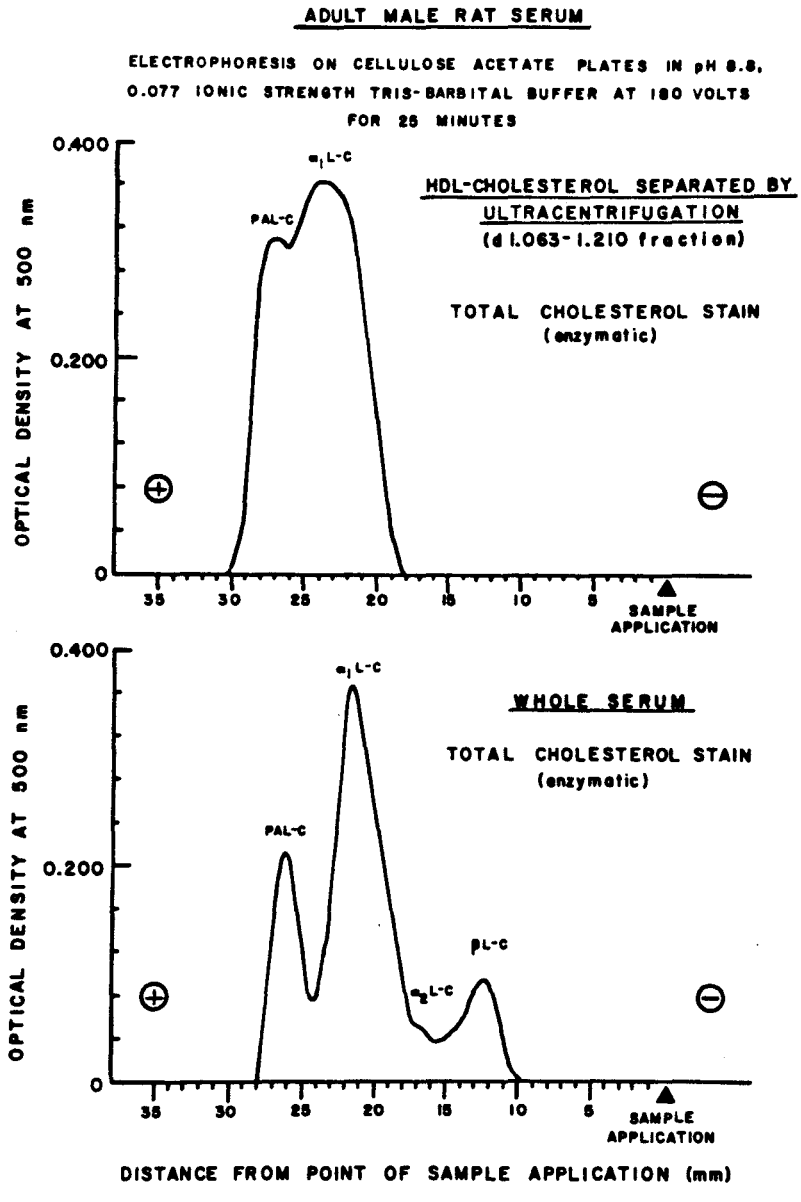

ADULT MALE RAT SERUM
ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.8, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER AT 180 VOLTS FOR 25 MINUTES
OPTICAL DENSITY AT 500 nm
0.400
0.200
0
α1L-C
PAL-C
HDL-CHOLESTEROL SEPARATED BY ULTRACENTRIFUGATION
(d 1.063-1.210 fraction)
TOTAL CHOLESTEROL STAIN
(enzymatic)
35 30 25 20 15 10 5
SAMPLE APPLICATION
WHOLE SERUM
TOTAL CHOLESTEROL STAIN
(enzymatic)
α2L-C
pL-C
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 7

Figure 8. Cellulose acetate electrophoresis of the high density fraction (greater than 1.063 g/ml) of adult male rat serum obtained by ultracentrifugation. A sample of the corresponding whole serum was stored at -20 °C during the time required to isolate the high density fraction, and was subsequently electrophoresed on the same plate. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol.)

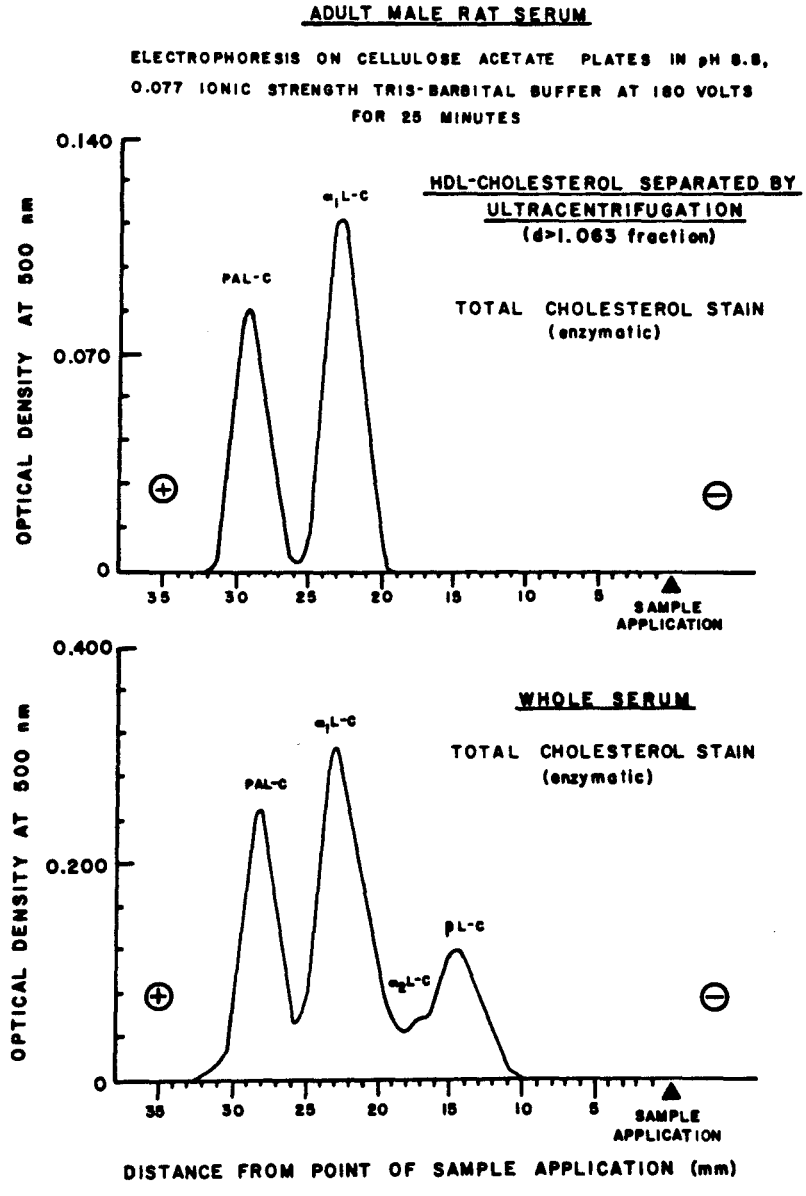

ADULT MALE RAT SERUM
ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.8, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER AT 180 VOLTS FOR 25 MINUTES
HDL-CHOLESTEROL SEPARATED BY ULTRACENTRIFUGATION (d>1.063 fraction)
TOTAL CHOLESTEROL STAIN (enzymatic)
PAL-C
α1L-C
OPTICAL DENSITY AT 500 nm
0.140
0.070
0
35 30 25 20 15 10 5
SAMPLE APPLICATION
WHOLE SERUM
TOTAL CHOLESTEROL STAIN (enzymatic)
PAL-C
α1L-C
α2L-C
βL-C
0.400
0.200
0
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 8

Figure 9. Cellulose acetate electrophoresis of the dialyzed supernate obtained after manganese heparin precipitation of adult male rat serum. A sample of the corresponding whole serum was stored at -20 °C during the time it took to dialyze the manganese heparin supernate against 0.15 M sodium chloride solution containing 0.05% EDTA at 4 °C, and was subsequently electrophoresed on the same plate. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol.)

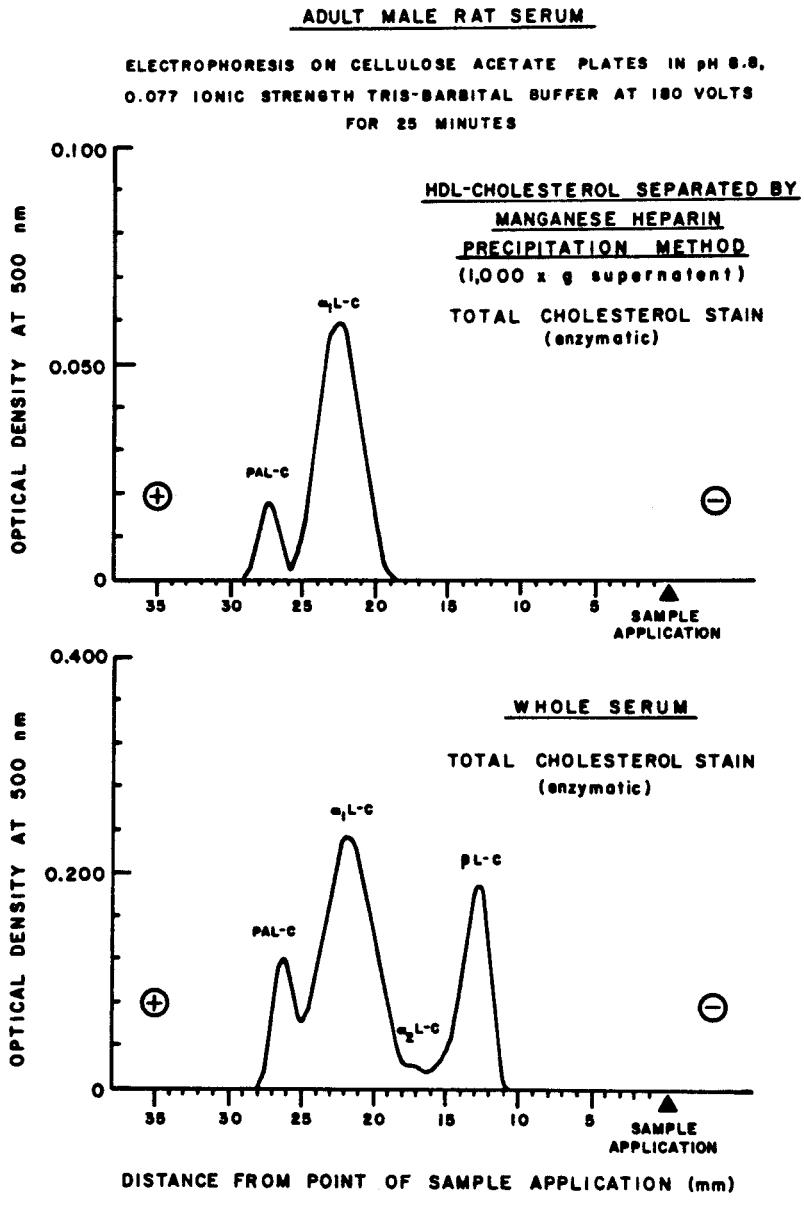

ADULT MALE RAT SERUM
ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.8, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER AT 180 VOLTS FOR 25 MINUTES
HDL-CHOLESTEROL SEPARATED BY MANGANESE HEPARIN PRECIPITATION METHOD (1,000 x g supernatant)
TOTAL CHOLESTEROL STAIN (enzymatic)
OPTICAL DENSITY AT 500 nm
0.100
0.050
0
PAL-C
α1L-C
35 30 25 20 15 10 5
SAMPLE APPLICATION
WHOLE SERUM
TOTAL CHOLESTEROL STAIN (enzymatic)
0.400
0.200
PAL-C
α1L-C
α2L-C
pL-C
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 9

Figure 10. Cellulose acetate electrophoresis of the supernate obtained after dextran sulfate precipitation of adult male rat serum. A sample of the corresponding whole serum was stored at -20 °C during the time required to isolate the high density fraction and was subsequently electrophoresed on the same plate. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol.)

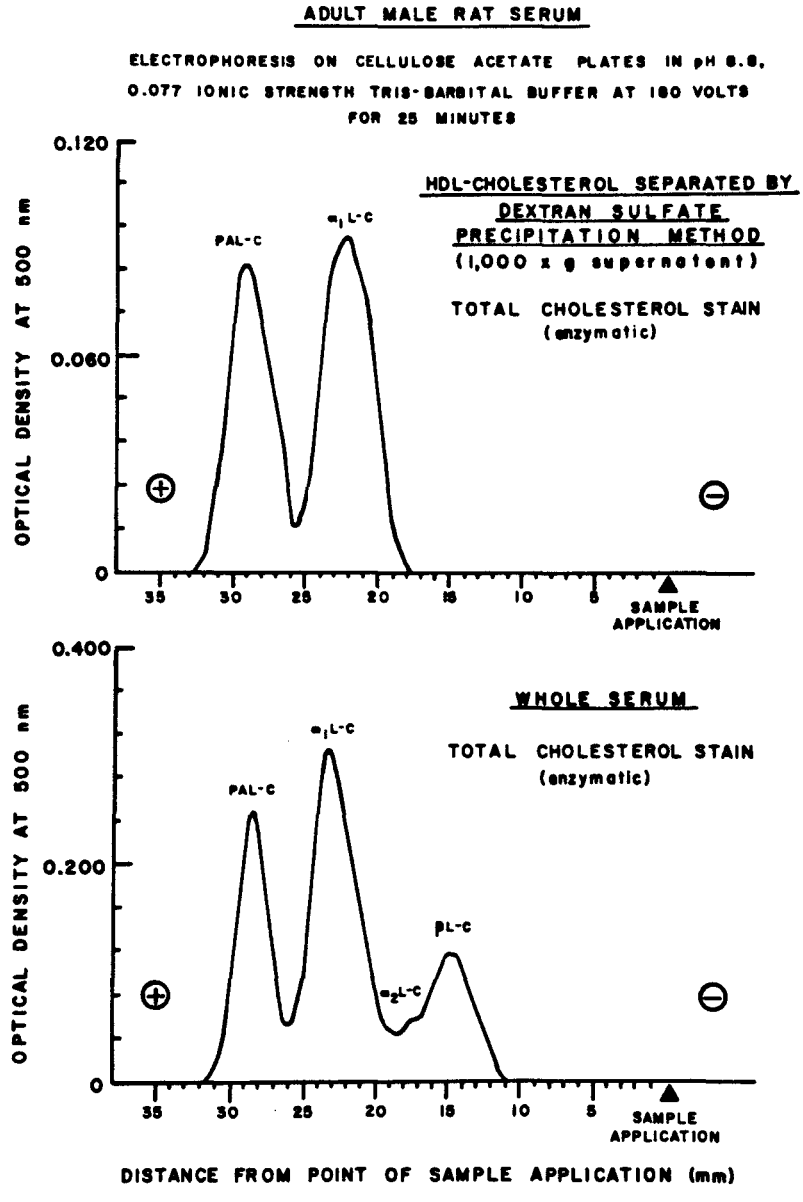

ADULT MALE RAT SERUM
ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.8, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER AT 180 VOLTS FOR 25 MINUTES
HDL-CHOLESTEROL SEPARATED BY DEXTRAN SULFATE PRECIPITATION METHOD (1,000 x g supernatant)
TOTAL CHOLESTEROL STAIN (enzymatic)
OPTICAL DENSITY AT 500 nm
0.120
0.060
0
PAL-C
α1L-C
35 30 25 20 15 10 5
SAMPLE APPLICATION
WHOLE SERUM
TOTAL CHOLESTEROL STAIN (enzymatic)
0.400
0.200
PAL-C
α1L-C
α2L-C
βL-C
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 10

Figure 11. Correlation of rat plasma high density lipoprotein-cholesterol levels determined by cellulose acetate electrophoresis versus manganese heparin precipitation method. The line was drawn with a slope of 1.02 obtained by linear regression analysis; y-intercept=9.30. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol.)

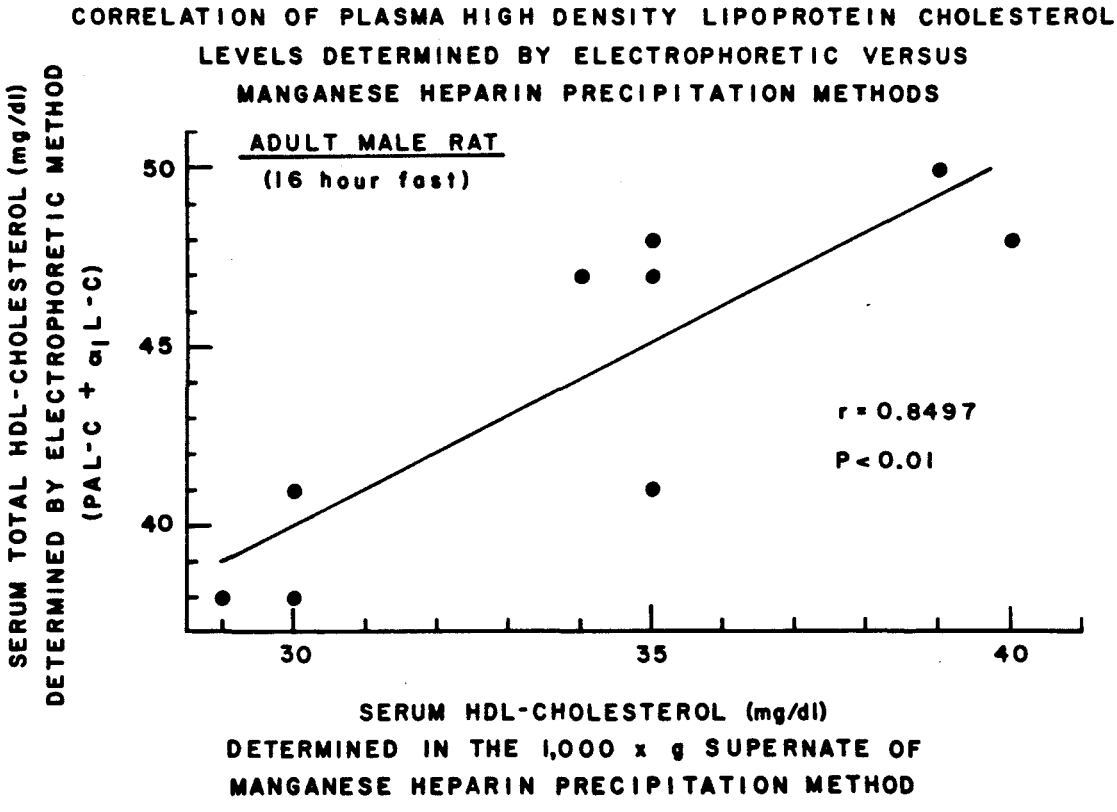

CORRELATION OF PLASMA HIGH DENSITY LIPOPROTEIN CHOLESTEROL
LEVELS DETERMINED BY ELECTROPHORETIC VERSUS
MANGANESE HEPARIN PRECIPITATION METHODS
ADULT MALE RAT
(16 hour fast)
SERUM TOTAL HDL-CHOLESTEROL (mg/dl)
DETERMINED BY ELECTROPHORETIC METHOD
(PAL-C + α1 L-C)
50
45
40
30
35
40
r = 0.8497
P < 0.01
SERUM HDL-CHOLESTEROL (mg/dl)
DETERMINED IN THE 1,000 x g SUPERNATE OF
MANGANESE HEPARIN PRECIPITATION METHOD

Figure 11

Figure 12. Correlation of rat plasma high density lipoprotein-cholesterol levels determined by cellulose acetate electrophoresis versus dextran sulfate precipitation method. The line was drawn with a slope of 1.08 obtained by linear regression analysis; y-intercept=15.65. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol.)

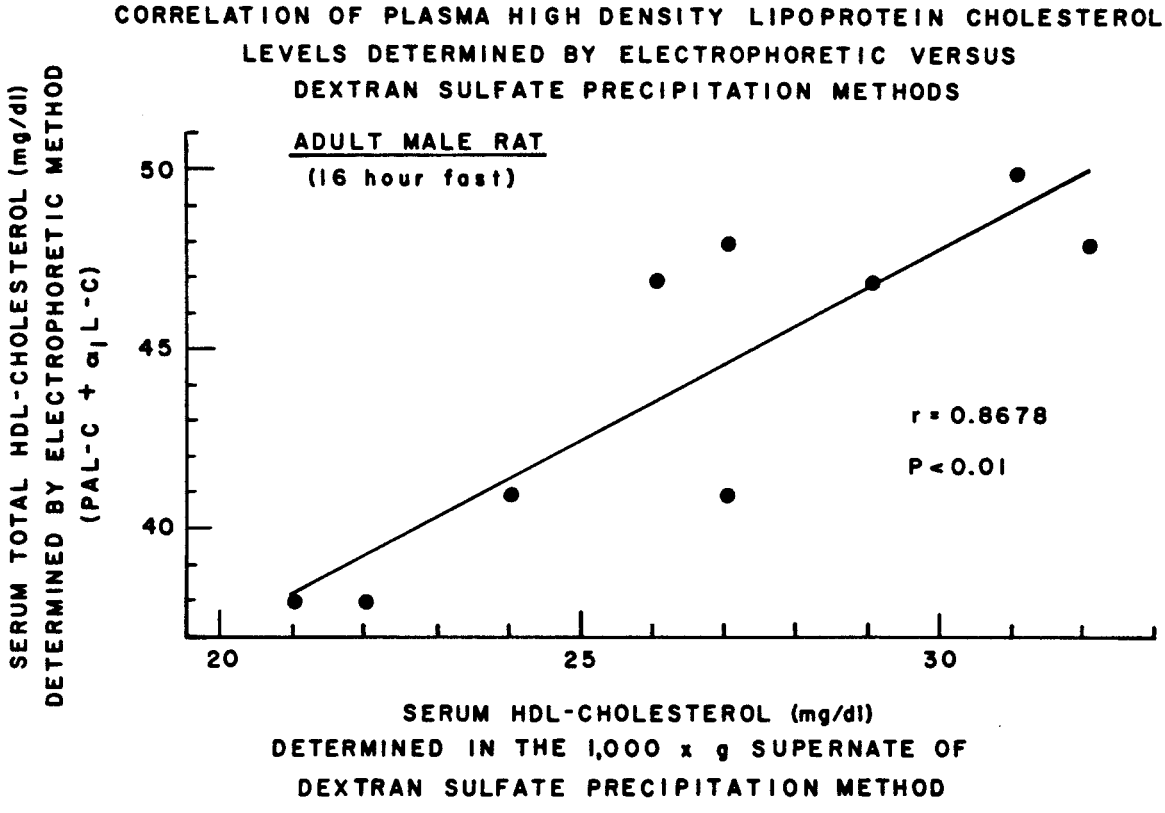

CORRELATION OF PLASMA HIGH DENSITY LIPOPROTEIN CHOLESTEROL
LEVELS DETERMINED BY ELECTROPHORETIC VERSUS
DEXTRAN SULFATE PRECIPITATION METHODS
ADULT MALE RAT
(16 hour fast)
r = 0.8678
P < 0.01
SERUM TOTAL HDL-CHOLESTEROL (mg/dl)
DETERMINED BY ELECTROPHORETIC METHOD
(PAL-C + α1 L-C)
50
45
40
20
25
30
SERUM HDL-CHOLESTEROL (mg/dl)
DETERMINED IN THE 1,000 x g SUPERNATE OF
DEXTRAN SULFATE PRECIPITATION METHOD

Figure 12

Figure 13. Correlation of rat plasma high density lipoprotein-cholesterol levels determined by dextran sulfate precipitation versus manganese heparin precipitation method. The line was drawn with a slope of 0.95 obtained by linear regression analysis; y-intercept=-5.7.

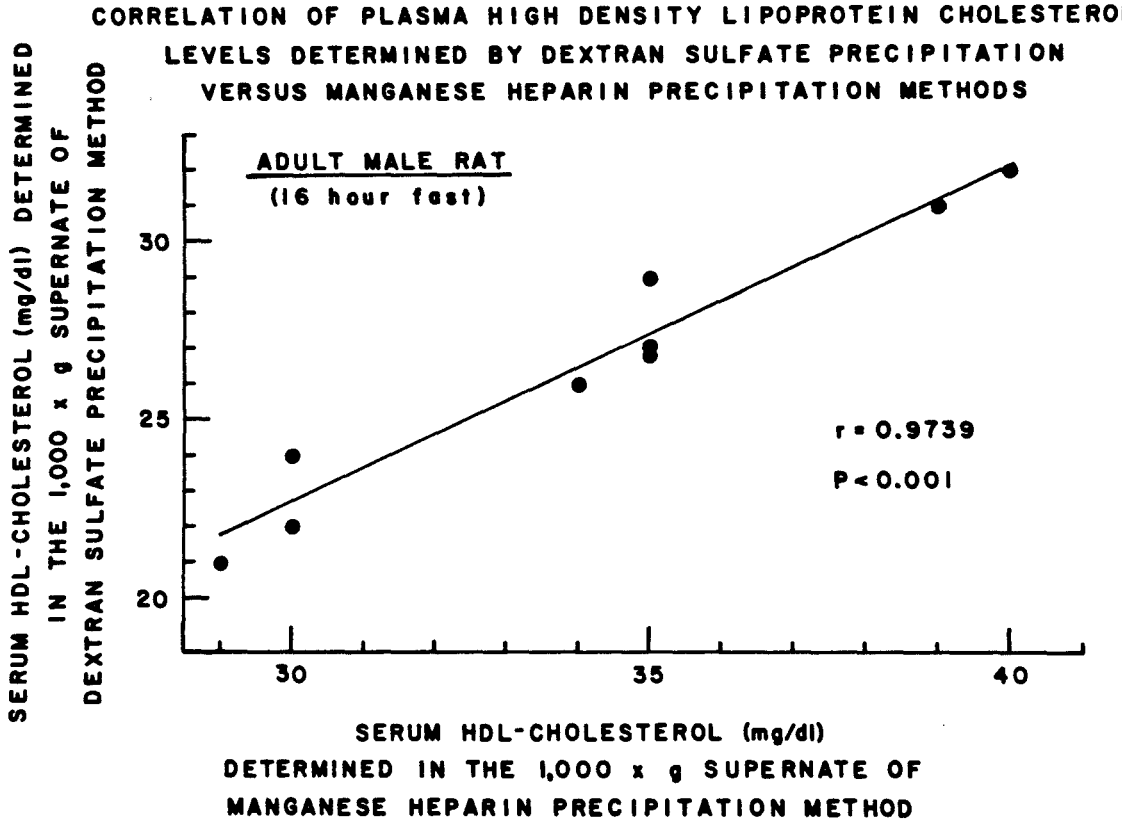

CORRELATION OF PLASMA HIGH DENSITY LIPOPROTEIN CHOLESTEROL
LEVELS DETERMINED BY DEXTRAN SULFATE PRECIPITATION
VERSUS MANGANESE HEPARIN PRECIPITATION METHODS
ADULT MALE RAT
(16 hour fast)
r = 0.9739
P < 0.001
SERUM HDL-CHOLESTEROL (mg/dl) DETERMINED IN THE 1,000 x g SUPERNATE OF DEXTRAN SULFATE PRECIPITATION METHOD
SERUM HDL-CHOLESTEROL (mg/dl) DETERMINED IN THE 1,000 x g SUPERNATE OF MANGANESE HEPARIN PRECIPITATION METHOD
20
25
30
30
35
40

Figure 13

Figure 14. Comparison of rat plasma high density lipoprotein-cholesterol (HDL-C) levels determined by cellulose acetate electrophoresis, manganese heparin precipitation and dextran sulfate precipitation. Statistical analysis was performed using one way analysis of variance followed by Duncan's new multiple range test. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol.)

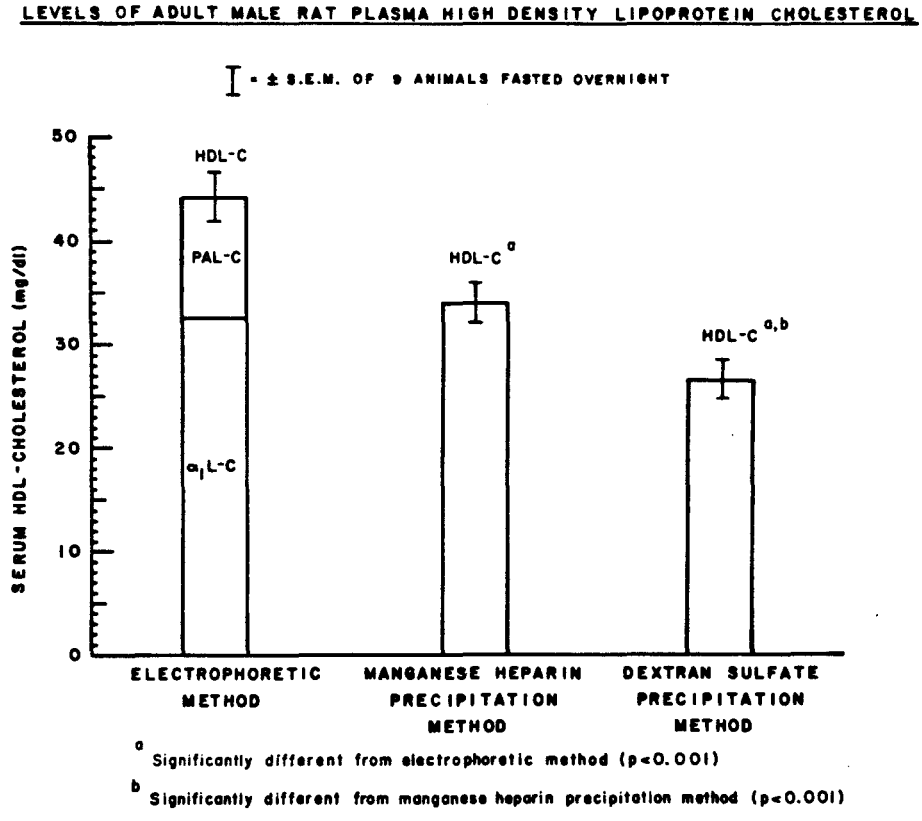

LEVELS OF ADULT MALE RAT PLASMA HIGH DENSITY LIPOPROTEIN CHOLESTEROL
I = ± S.E.M. OF 9 ANIMALS FASTED OVERNIGHT
SERUM HDL-CHOLESTEROL (mg/dl)
50
40
30
20
10
0
HDL-C
PAL-C
α_1L-C
HDL-C [a]
HDL-C [a,b]
ELECTROPHORETIC METHOD
MANGANESE HEPARIN PRECIPITATION METHOD
DEXTRAN SULFATE PRECIPITATION METHOD
[a] Significantly different from electrophoretic method (p<0.001)
[b] Significantly different from manganese heparin precipitation method (p<0.001)

Figure 14

Figure 15. Kinetic changes in adult male rat plasma lipoprotein-total cholesterol fractions separated by cellulose acetate electrophoresis during a 4 hour *in vitro* incubation of plasma at 37 °C. (PAL-C is prealbumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol.)

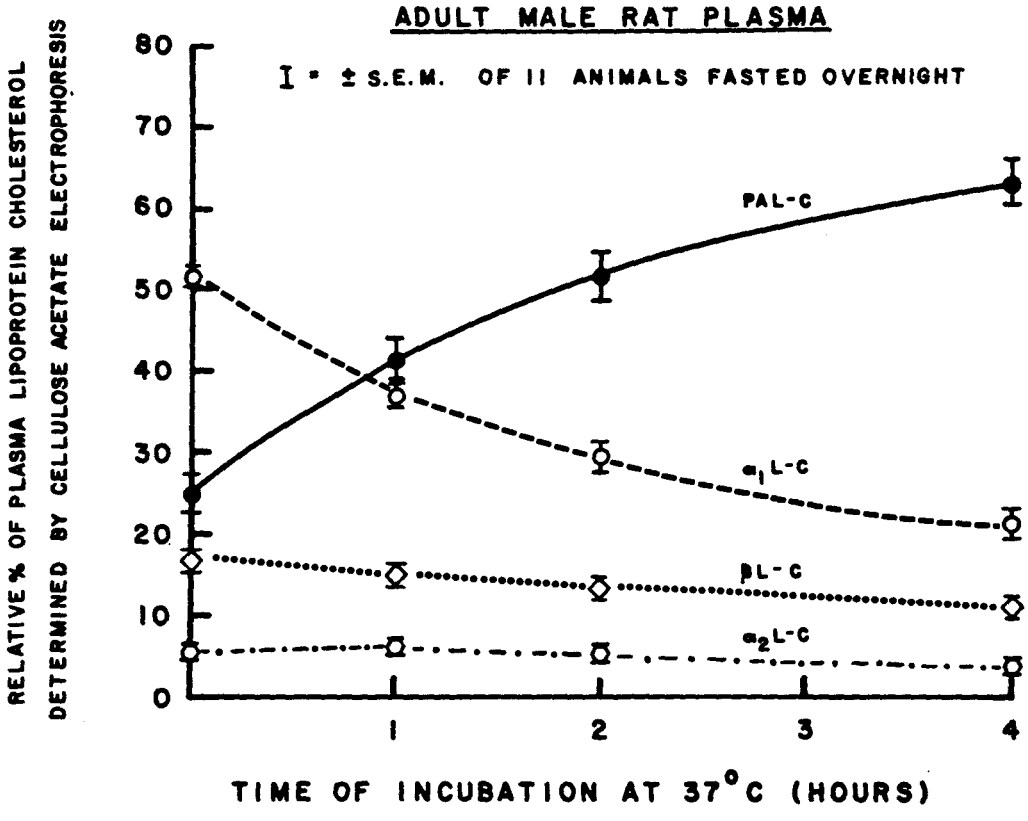

ADULT MALE RAT PLASMA
I = ± S.E.M. OF 11 ANIMALS FASTED OVERNIGHT
RELATIVE % OF PLASMA LIPOPROTEIN CHOLESTEROL DETERMINED BY CELLULOSE ACETATE ELECTROPHORESIS
80
70
60
50
40
30
20
10
0
PAL-C
α1L-C
βL-C
α2L-C
1
2
3
4
TIME OF INCUBATION AT 37°C (HOURS)

Figure 15

Figure 16. Comparisons of rat plasma lipoprotein-cholesterol profiles after a 1 hour and 4 hour *in vitro* incubation at 37 °C. (PAL-C is pre-albumin lipoprotein-cholesterol; α_1L-C is alpha$_1$ lipoprotein-cholesterol; α_2L-C is alpha$_2$ lipoprotein-cholesterol; βL-C is beta lipoprotein-cholesterol.)

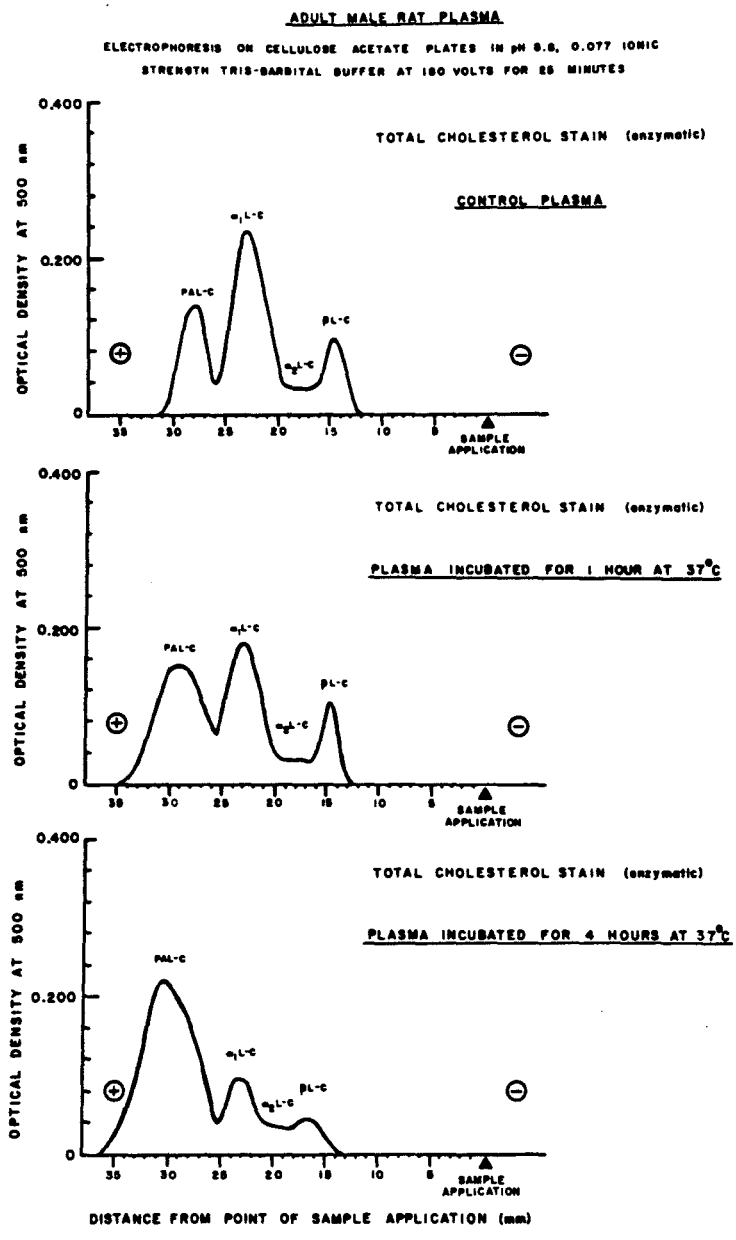

ADULT MALE RAT PLASMA
ELECTROPHORESIS ON CELLULOSE ACETATE PLATES IN pH 8.6, 0.077 IONIC STRENGTH TRIS-BARBITAL BUFFER AT 180 VOLTS FOR 25 MINUTES
TOTAL CHOLESTEROL STAIN (enzymatic)
CONTROL PLASMA
TOTAL CHOLESTEROL STAIN (enzymatic)
PLASMA INCUBATED FOR 1 HOUR AT 37°C
TOTAL CHOLESTEROL STAIN (enzymatic)
PLASMA INCUBATED FOR 4 HOURS AT 37°C
OPTICAL DENSITY AT 500 nm
0.400
0.200
0
PAL-C
α1L-C
α2L-C
βL-C
35 30 25 20 15 10 5
SAMPLE APPLICATION
DISTANCE FROM POINT OF SAMPLE APPLICATION (mm)

Figure 16

Figure 17. Changes in lipoprotein-cholesterol fractions separated by cellulose acetate electrophoresis following a 4 hour 37 °C *in vitro* incubation of adult male rat plasma. Statistical analysis between the initial lipoprotein-cholesterol concentrations and those after four hours of incubation was performed using a paired Student's t-test. (PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; α_2L is alpha$_2$ lipoprotein; βL is beta lipoprotein.)

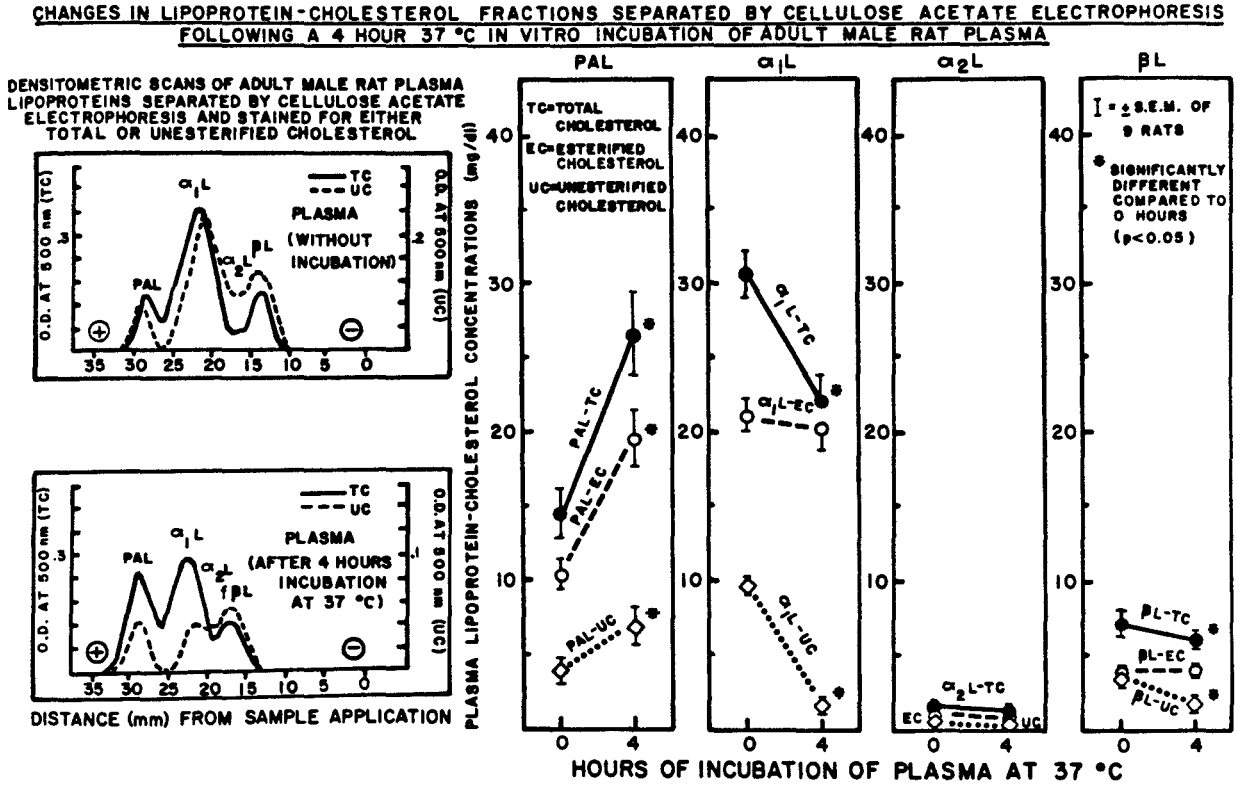

CHANGES IN LIPOPROTEIN-CHOLESTEROL FRACTIONS SEPARATED BY CELLULOSE ACETATE ELECTROPHORESIS FOLLOWING A 4 HOUR 37 °C IN VITRO INCUBATION OF ADULT MALE RAT PLASMA
DENSITOMETRIC SCANS OF ADULT MALE RAT PLASMA LIPOPROTEINS SEPARATED BY CELLULOSE ACETATE ELECTROPHORESIS AND STAINED FOR EITHER TOTAL OR UNESTERIFIED CHOLESTEROL
O.D. AT 500 nm (TC)
O.D. AT 500 nm (UC)
TC
UC
PLASMA (WITHOUT INCUBATION)
PLASMA (AFTER 4 HOURS INCUBATION AT 37 °C)
PAL
α1L
α2L
βL
DISTANCE (mm) FROM SAMPLE APPLICATION
PLASMA LIPOPROTEIN-CHOLESTEROL CONCENTRATIONS (mg/dl)
TC=TOTAL CHOLESTEROL
EC=ESTERIFIED CHOLESTEROL
UC=UNESTERIFIED CHOLESTEROL
± S.E.M. OF 9 RATS
* SIGNIFICANTLY DIFFERENT COMPARED TO 0 HOURS (p<0.05)
PAL-TC
PAL-EC
PAL-UC
α1L-TC
α1L-EC
α1L-UC
α2L-TC
EC
UC
βL-TC
βL-EC
βL-UC
HOURS OF INCUBATION OF PLASMA AT 37 °C

Figure 17

Figure 18. Changes in lipoprotein-cholesterol fractions separated by cellulose acetate electrophoresis following a 4 hour 37 °C *in vitro* incubation of adult male rat plasma in the presence of an LCAT inhibitor (2.0 mM PCMPS). Statistical analysis between the initial lipoprotein-cholesterol concentrations and those after four hours of incubation was performed using a paired Student's t-test. (PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; α_2L is alpha$_2$ lipoprotein; βL is beta lipoprotein.)

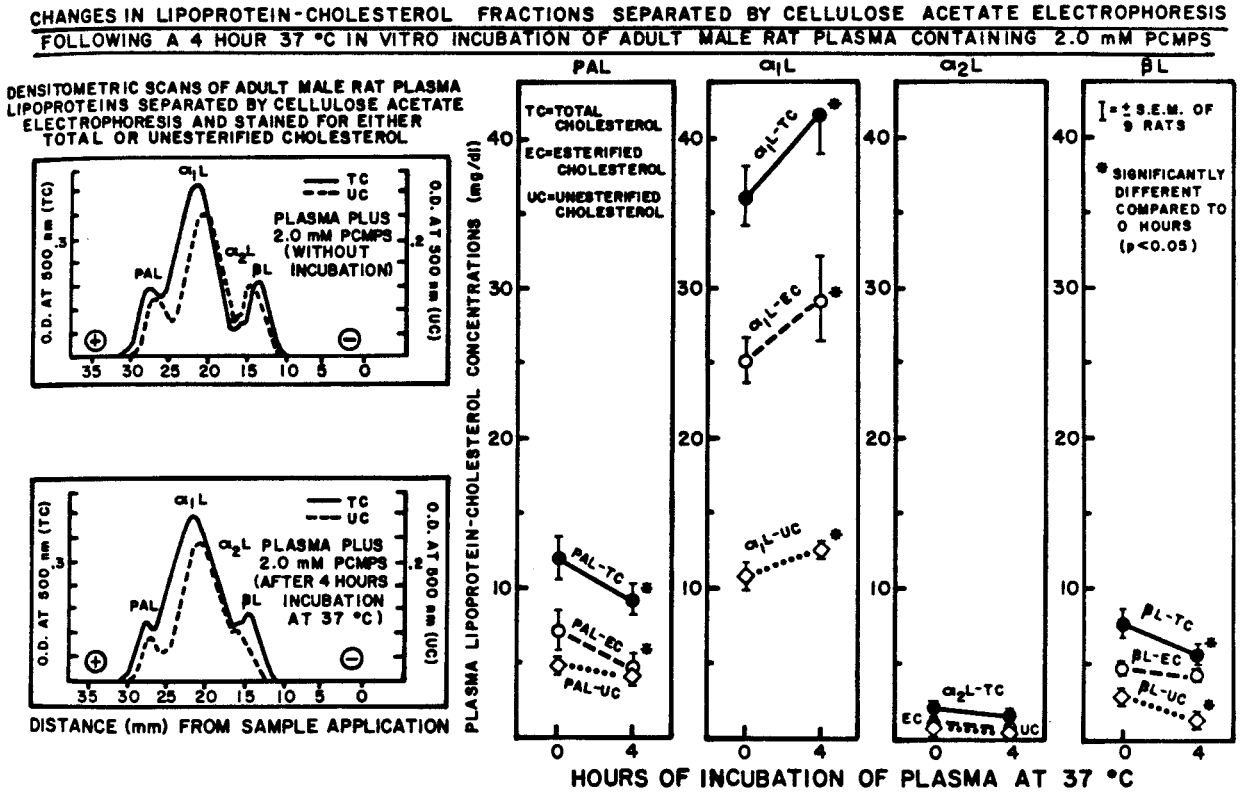

CHANGES IN LIPOPROTEIN-CHOLESTEROL FRACTIONS SEPARATED BY CELLULOSE ACETATE ELECTROPHORESIS
FOLLOWING A 4 HOUR 37 °C IN VITRO INCUBATION OF ADULT MALE RAT PLASMA CONTAINING 2.0 mM PCMPS
DENSITOMETRIC SCANS OF ADULT MALE RAT PLASMA LIPOPROTEINS SEPARATED BY CELLULOSE ACETATE ELECTROPHORESIS AND STAINED FOR EITHER TOTAL OR UNESTERIFIED CHOLESTEROL
O.D. AT 600 nm (TC)
O.D. AT 600 nm (UC)
α1L
α2L
PAL
βL
TC
UC
PLASMA PLUS 2.0 mM PCMPS (WITHOUT INCUBATION)
PLASMA PLUS 2.0 mM PCMPS (AFTER 4 HOURS INCUBATION AT 37 °C)
35 30 25 20 15 10 5 0
DISTANCE (mm) FROM SAMPLE APPLICATION
PLASMA LIPOPROTEIN-CHOLESTEROL CONCENTRATIONS (mg/dl)
TC=TOTAL CHOLESTEROL
EC=ESTERIFIED CHOLESTEROL
UC=UNESTERIFIED CHOLESTEROL
PAL-TC
PAL-EC
PAL-UC
α1L-TC
α1L-EC
α1L-UC
α2L-TC
EC
UC
βL-TC
βL-EC
βL-UC
± S.E.M. OF 9 RATS
* SIGNIFICANTLY DIFFERENT COMPARED TO 0 HOURS (p<0.05)
HOURS OF INCUBATION OF PLASMA AT 37 °C

Figure 18

Figure 19. Changes in esterified to unesterified cholesterol ratios of lipoprotein fractions separated by cellulose acetate electrophoresis following a 4 hour 37 °C *in vitro* incubation of adult male rat plasma. Statistical analysis between the initial lipoprotein-esterified cholesterol to unesterified cholesterol ratios and those after four hours of incubation was performed using a paired Student's t-test. (PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; α_2L is alpha$_2$ lipoprotein; βL is beta lipoprotein.)

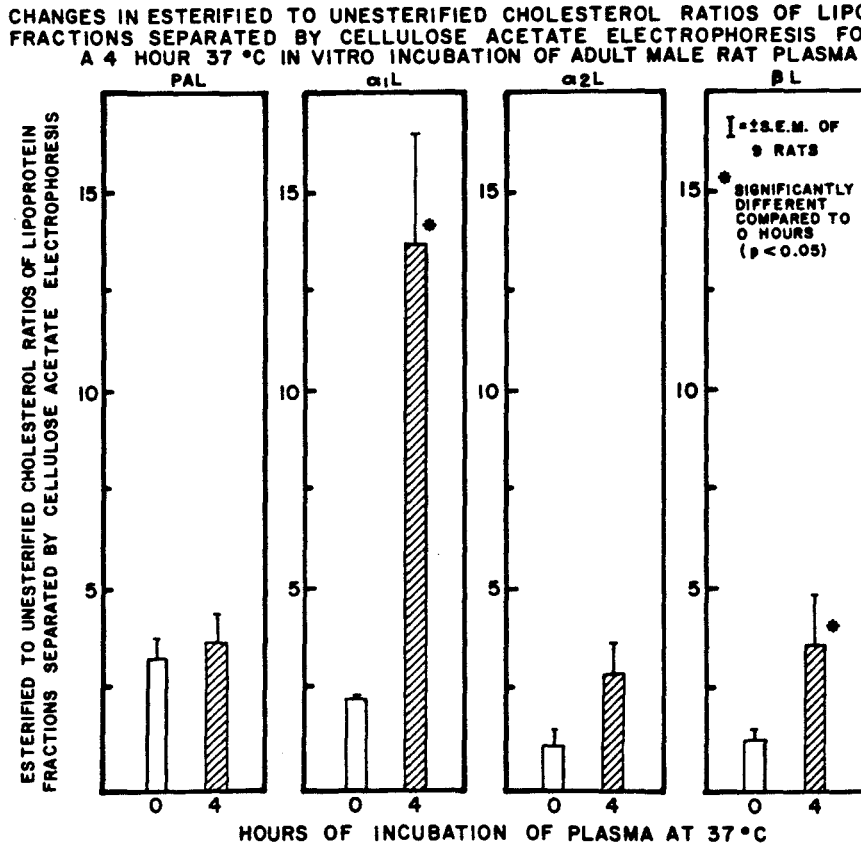

CHANGES IN ESTERIFIED TO UNESTERIFIED CHOLESTEROL RATIOS OF LIPOPROTEIN FRACTIONS SEPARATED BY CELLULOSE ACETATE ELECTROPHORESIS FOLLOWING A 4 HOUR 37 °C IN VITRO INCUBATION OF ADULT MALE RAT PLASMA
PAL
α1L
α2L
βL
I = ±S.E.M. OF 9 RATS
* SIGNIFICANTLY DIFFERENT COMPARED TO 0 HOURS (p < 0.05)
ESTERIFIED TO UNESTERIFIED CHOLESTEROL RATIOS OF LIPOPROTEIN FRACTIONS SEPARATED BY CELLULOSE ACETATE ELECTROPHORESIS
15
10
5
0
4
HOURS OF INCUBATION OF PLASMA AT 37 °C

Figure 19

Figure 20. Changes in esterified to unesterified cholesterol ratios of lipoprotein fractions separated by cellulose acetate electrophoresis following a 4 hour 37 °C *in vitro* incubation of adult male rat plasma in the presence of an LCAT inhibitor (2.0 mM PCMPS). Statistical analysis between the initial lipoprotein-esterified cholesterol to unesterified cholesterol ratios and those after four hours of incubation was performed using a paired Student's t-test. (PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; α_2L is alpha$_2$ lipoprotein; βL is beta lipoprotein.)

CHANGES IN ESTERIFIED TO UNESTERIFIED CHOLESTEROL RATIOS OF LIPOPROTEIN FRACTIONS SEPARATED BY CELLULOSE ACETATE ELECTROPHORESIS FOLLOWING A 4 HOUR 37 °C IN VITRO INCUBATION OF ADULT MALE RAT PLASMA CONTAINING 2.0 mM PCMPS

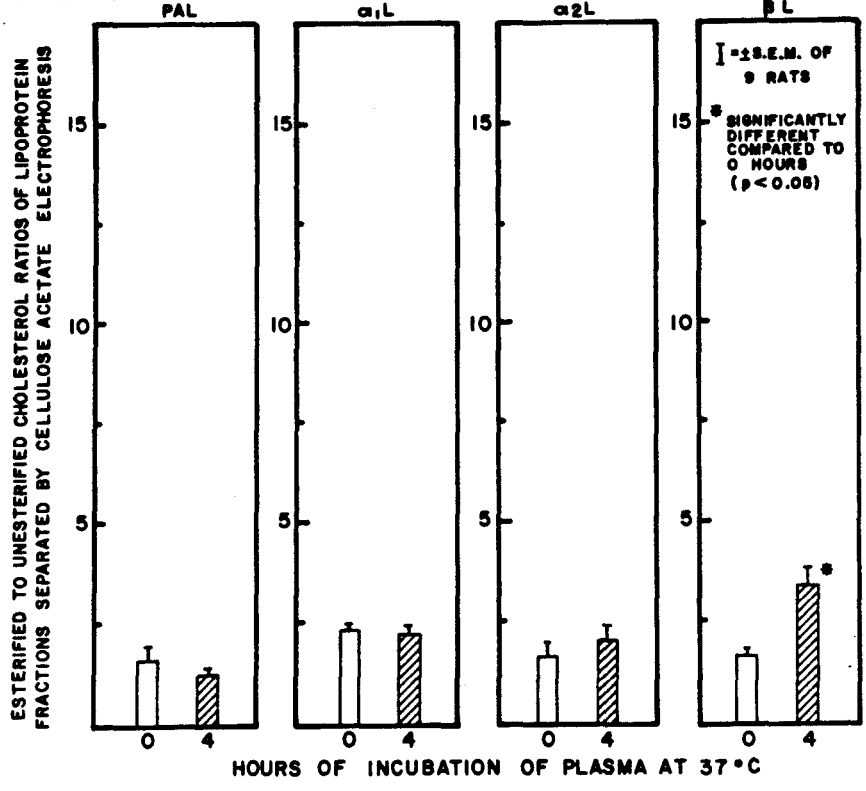


Figure 20

Figure 21. Effect of 17 α-ethinyl estradiol on total cholesterol concentration of adult male rat plasma. Rats were administered daily doses of 17 α-ethinyl estradiol subcutaneously for 4 days and sacrificed on day 5. All plasma total cholesterol concentrations of rats given 17 α-ethinyl estradiol were statistically significantly lower than the control animals. The control value represents the mean plasma total cholesterol concentration of 5 rats. The 0.05 mg/kg dose of 17 α-ethinyl estradiol represents the mean plasma total cholesterol concentration of 2 rats. All other points represent the mean plasma total cholesterol concentration of 4 rats. Statistical analysis was performed using one way analysis of variance followed by Duncan's new multiple range test.

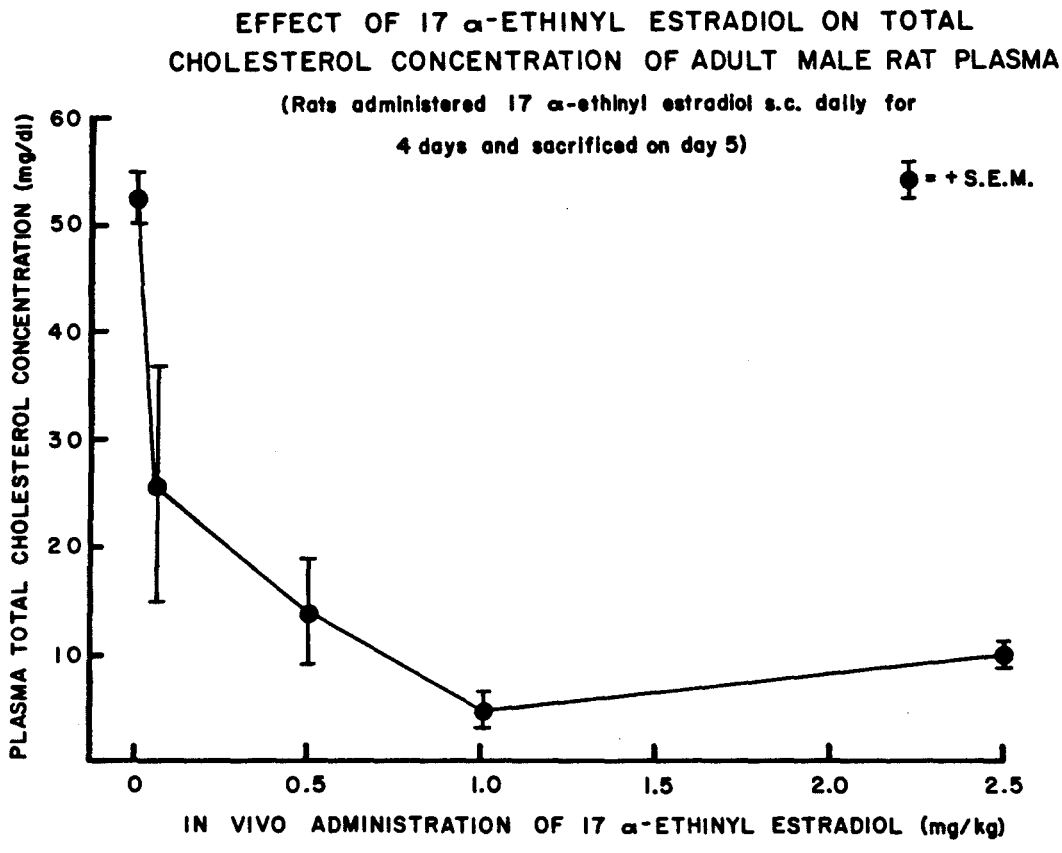

EFFECT OF 17 α-ETHINYL ESTRADIOL ON TOTAL
CHOLESTEROL CONCENTRATION OF ADULT MALE RAT PLASMA
(Rats administered 17 α-ethinyl estradiol s.c. daily for
4 days and sacrificed on day 5)
= + S.E.M.
PLASMA TOTAL CHOLESTEROL CONCENTRATION (mg/dl)
60
50
40
30
20
10
0
0.5
1.0
1.5
2.0
2.5
IN VIVO ADMINISTRATION OF 17 α-ETHINYL ESTRADIOL (mg/kg)

Figure 21

Figure 22. Effects of *in vivo* administration of 17 α-ethinyl estradiol on lipoprotein-total cholesterol concentration before and after a 4 hour 37 °C *in vitro* incubation of adult male rat plasma. Rats were administered daily doses of 17 α-ethinyl estradiol subcutaneously for 4 days and sacrificed on day 5. Statistical analysis was performed using one way analysis of variance followed by Duncan's new multiple range test. (PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; α_2L is alpha$_2$ lipoprotein; βL is beta lipoprotein.)

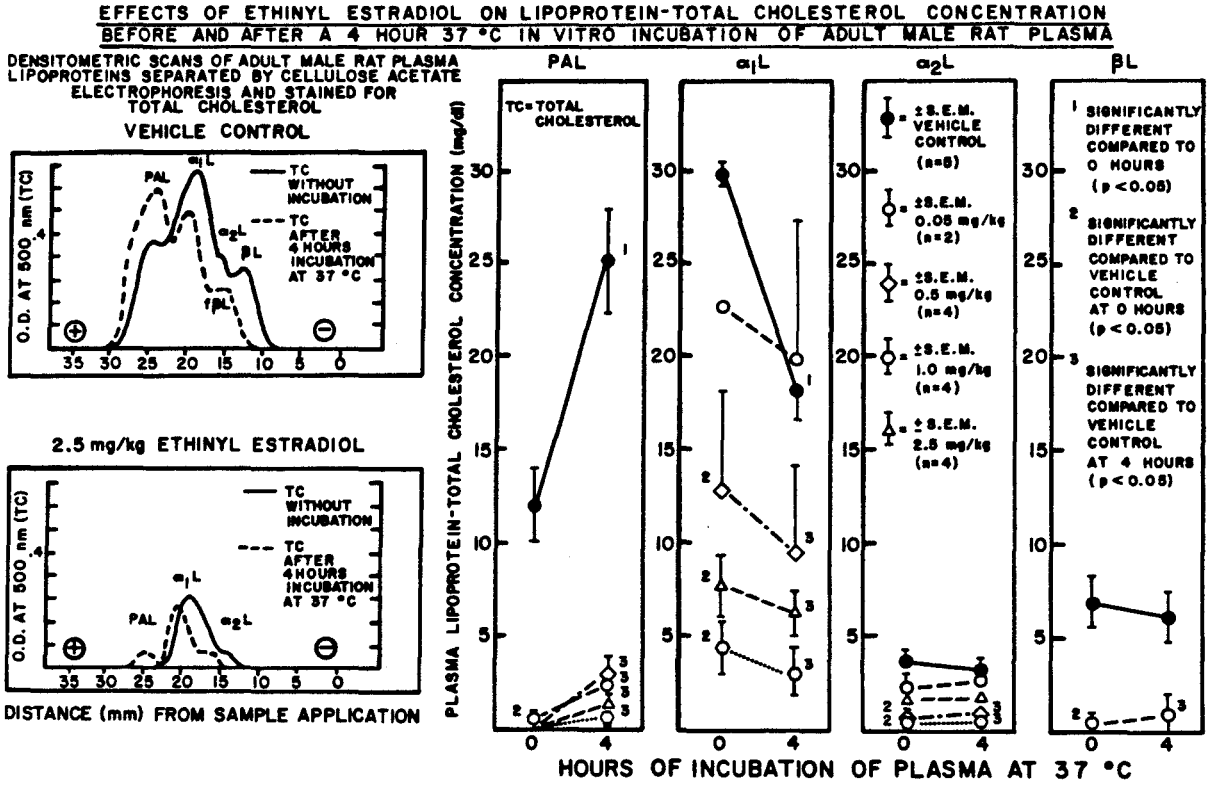
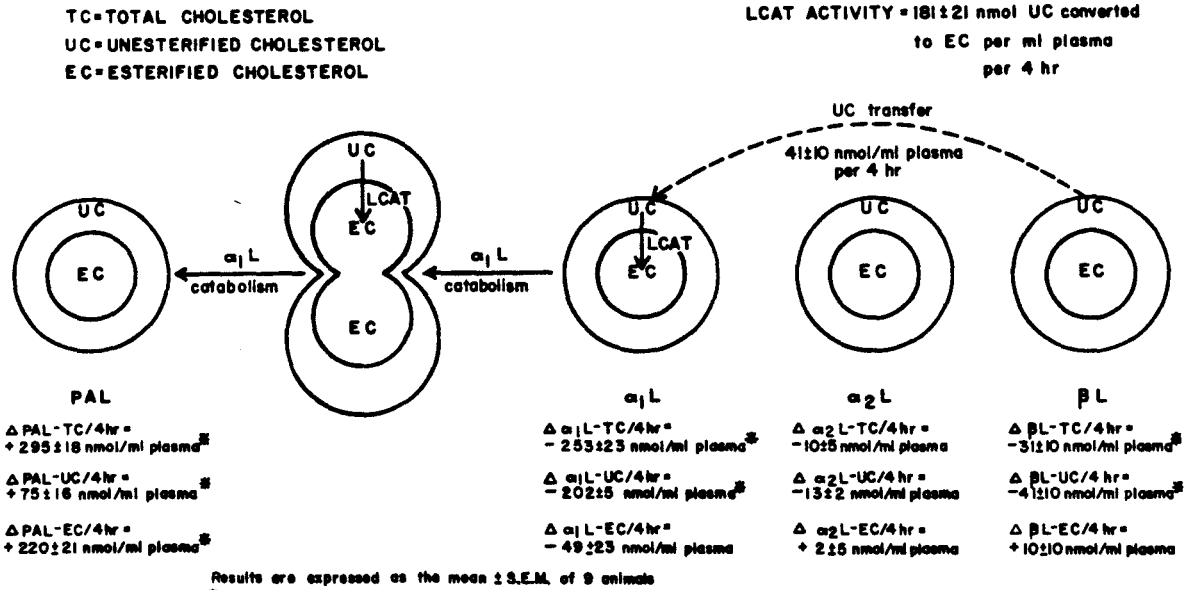

EFFECTS OF ETHINYL ESTRADIOL ON LIPOPROTEIN-TOTAL CHOLESTEROL CONCENTRATION
BEFORE AND AFTER A 4 HOUR 37 °C IN VITRO INCUBATION OF ADULT MALE RAT PLASMA
DENSITOMETRIC SCANS OF ADULT MALE RAT PLASMA LIPOPROTEINS SEPARATED BY CELLULOSE ACETATE ELECTROPHORESIS AND STAINED FOR TOTAL CHOLESTEROL
VEHICLE CONTROL
2.5 mg/kg ETHINYL ESTRADIOL
O.D. AT 500 nm (TC)
PAL
α1L
α2L
βL
TC WITHOUT INCUBATION
TC AFTER 4 HOURS INCUBATION AT 37 °C
DISTANCE (mm) FROM SAMPLE APPLICATION
PAL
α1L
α2L
βL
TC=TOTAL CHOLESTEROL
PLASMA LIPOPROTEIN-TOTAL CHOLESTEROL CONCENTRATION (mg/dl)
±S.E.M. VEHICLE CONTROL (n=8)
±S.E.M. 0.05 mg/kg (n=2)
±S.E.M. 0.5 mg/kg (n=4)
±S.E.M. 1.0 mg/kg (n=4)
±S.E.M. 2.5 mg/kg (n=4)
1 SIGNIFICANTLY DIFFERENT COMPARED TO 0 HOURS (p<0.05)
2 SIGNIFICANTLY DIFFERENT COMPARED TO VEHICLE CONTROL AT 0 HOURS (p<0.05)
3 SIGNIFICANTLY DIFFERENT COMPARED TO VEHICLE CONTROL AT 4 HOURS (p<0.05)
HOURS OF INCUBATION OF PLASMA AT 37 °C

Figure 22

Figure 23. Proposed intravascular lipoprotein catabolic transformation of adult male rat alpha$_1$ lipoprotein following a 4 hour 37 °C *in vitro* incubation of plasma. Statistical analysis between the initial lipoprotein-cholesterol concentrations and those after four hours of incubation was performed using a paired Student's t-test. (PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; α_2L is alpha$_2$ lipoprotein; βL is beta lipoprotein.)

PROPOSED INTRAVASCULAR LIPOPROTEIN CATABOLIC TRANSFORMATION OF ADULT MALE RAT ALPHA$_1$ LIPOPROTEIN FOLLOWING A 4 HOUR 37 °C IN VITRO INCUBATION OF PLASMA



Results are expressed as the mean ± S.E.M. of 9 animals

* Significant change in incubated versus non-incubated value ($p < 0.05$)

Figure 23

Figure 24. Inhibition of intravascular lipoprotein catabolic transformation of adult male rat alpha$_1$ lipoprotein following a 4 hour 37 °C *in vitro* incubation of plasma containing an LCAT inhibitor (2.0 mM PCMPS). Statistical analysis between the initial lipoprotein-cholesterol concentrations and those after four hours of incubation was performed using a paired Student's t-test. (PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; α_2L is alpha$_2$ lipoprotein; βL is beta lipoprotein.)

INHIBITION OF INTRAVASCULAR LIPOPROTEIN CATABOLIC TRANSFORMATION OF ADULT MALE RAT ALPHA$_1$ LIPOPROTEIN FOLLOWING A 4 HOUR 37 °C IN VITRO INCUBATION OF PLASMA CONTAINING 2.0 mM PCMPS

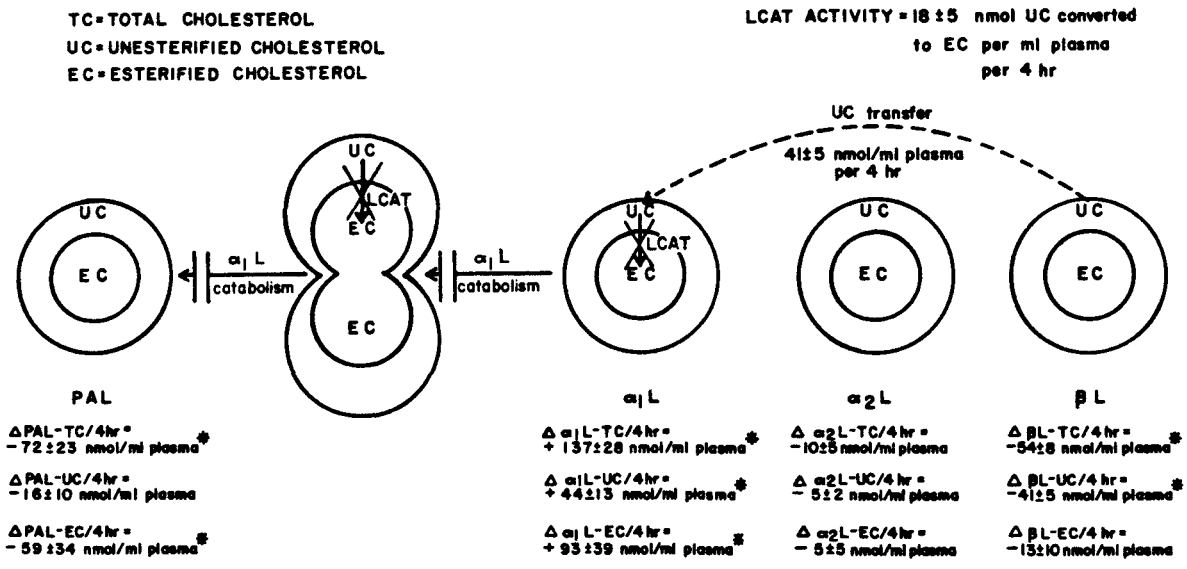
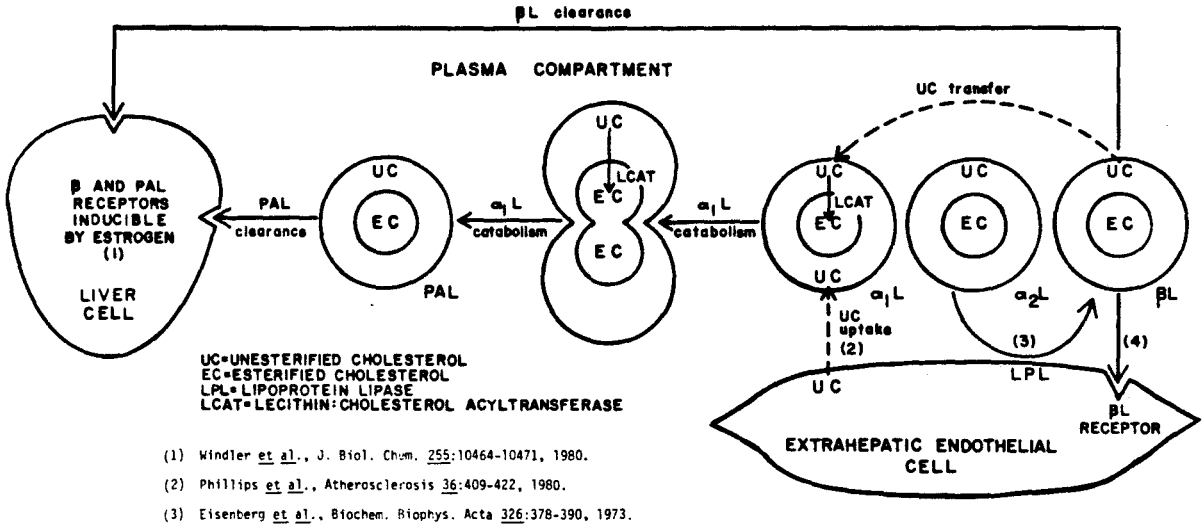


Figure 24

Figure 25. Proposed in vivo mechanism for cholesterol clearance by circulating plasma lipoproteins in the adult male rat. (PAL is prealbumin lipoprotein; α_1L is alpha$_1$ lipoprotein; α_2L is alpha$_2$ lipoprotein; βL is beta lipoprotein.)

PROPOSED IN VIVO MECHANISM FOR CHOLESTEROL CLEARANCE BY CIRCULATING PLASMA LIPOPROTEINS IN THE ADULT MALE RAT



(1) Windler et al., J. Biol. Chem. 255:10464-10471, 1980.

(2) Phillips et al., Atherosclerosis 36:409-422, 1980.

(3) Eisenberg et al., Biochem. Biophys. Acta 326:378-390, 1973.

(4) Brown et al., Science 212:628-635, 1981.

Figure 25

CHAPTER VIII

TABLES

TABLE 1

DISTRIBUTION AND COMPOSITION OF LIPOPROTEINS IN VARIOUS MAMMALS*

Species	Serum			Density <1.019 gm/ml				Density 1.019-1.063 gm/ml				Density >1.063		
	TC	PL	$\frac{TC}{PL}$	TC	PL	Protein	$\frac{TC}{PL}$	TC	PL	Protein	$\frac{TC}{PL}$	TC	PL	$\frac{TC}{PL}$
	(mg/100 ml)			(mg/100 ml)				(mg/100 ml)				(mg/100 ml)		
Mean values young adult humans	179	226	0.79	23	28	17	0.82	103	74	60	1.39	49	123	0.40
Rabbit	41	93	0.44	15	18	14	0.83	9	11	12	0.83	17	69	0.25
Rat	50	114	0.44	6	17	12	0.35	10	13	17	0.77	31	78	0.40
Pig	106	161	0.66	8	12	10	0.67	51	37	36	1.4	41	102	0.40
Hamster	121	269	0.45	25	44	21	0.57	29	34	22	0.85	61	176	0.35
Dog	150	362	0.41	2	10	9	0.20	10	19	17	0.53	127	325	0.39
Monkey	173	295	0.59	17	20	13	0.85	64	64	52	1.0	80	206	0.39

*From Havel et al., 1955. Abbreviations: TC = total cholesterol, PL = phospholipid

TABLE 2

DISTRIBUTION AND COMPOSITION OF HUMAN AND RAT SERUM LIPOPROTEINS SEPARATED BY SEQUENTIAL ULTRACENTRIFUGATION*

Species	Density (g/ml)	Cholesterol Ester	Unesterified Cholesterol	Total Cholesterol	Triglyceride	Phospholipid	Protein
		(Mean percent of lipoprotein weight)					
Human	Chylomicrons	6.0	3.1	9.1	81.3	7.0	2.5
Rat	<1.006 (VLDL)	4.2	8.9	13.1	61.1	12.0	13.8
Human	<1.019 (VLDL & IDL)	16.2	6.0	18.2	51.8	17.9	7.1
Rat	1.006-1.019 (IDL)	12.5	18.1	30.6	41.1	12.0	16.3
Human	1.019-1.063 (LDL)	39.4	7.5	46.9	9.3	23.1	20.7
Rat	1.019-1.063 (LDL)	15.0	19.1	34.1	12.2	27.2	26.5
Human	1.063-1.210 (HDL)	17.4	2.0	19.4	8.1	26.1	46.4
Rat	1.063-1.210 (HDL)	15.0	14.8	29.8	2.8	26.0	41.4

*Human data from Bragdon et al., 1956 and Rat data from Pasquali-Ronchetti et al., 1975.

TABLE 3

SERUM LIPOPROTEIN-CHOLESTEROL VALUES OF NORMAL, NONFASTING ANIMALS

	n	Total Cholesterol mg/dl (Mean ± SD)	PAL-C mg/dl (Mean ± SD)	α_1L-C mg/dl (Mean ± SD)	α_2L-C mg/dl (Mean ± SD)	βL-C mg/dl (Mean ± SD)	$\frac{\text{LDL-C}^*}{\text{HDL-C}}$	$\frac{\text{TOTAL-C}^*}{\text{HDL-C}}$
Adult Male Rat Serum	15	59 ± 11	15 ± 4	33 ± 7	3 ± 1	8 ± 2	0.17	1.23
Adult Male Mouse Serum	6	127 ± 20	0	109 ± 15	7 ± 3	11 ± 5	0.10	1.16
Adult Male Rabbit Serum	10	34 ± 8	0	20 ± 6	3 ± 2	11 ± 3	0.55	1.70
Adult Male Dog Serum	3	139 ± 15	0	116 ± 11	12 ± 2	11 ± 3	0.09	1.20
Adult Male Monkey Serum	3	147 ± 25	0	89 ± 30	12 ± 5	46 ± 5	0.52	1.65
Adult Male Human Serum (21-39 yrs)	13	183 ± 47	0	44 ± 9	12 ± 6	127 ± 46	3.05	4.33

* To calculate the comparative ratios (2), LDL-C is assumed to be equivalent to βL-C and HDL-C is assumed to be equivalent to PAL-C + α_1L-C.

TABLE 4

CHANGES IN ELECTROPHORETIC MOBILITY OF ADULT MALE RAT PLASMA LIPOPROTEIN FRACTIONS FOLLOWING A FOUR HOUR 37 °C IN VITRO INCUBATION AND COMPARED TO RAT PLASMA INCUBATED WITH AN LCAT INHIBITOR (2.0 mM PCMPS)[a].

	PAL	α_1L	α_2L	βL
	Distance from Sample Application (millimeters)			
Electrophoretic Mobility Rat Plasma t_0	27.0 ± 0.4	21.2 ± 0.2	16.0 ± 0.2	12.8 ± 0.1
Electorphoretic Mobility Rat Plasma t_4	27.1 ± 0.8	21.5 ± 0.4	17.9 ± 0.5[b]	16.1 ± 0.4[b]
% Change in Electrophoretic Mobility t_4 Compared to Rat Plasma t_0	0.4%	1.4%	11.9%	25.8%
Electrophoretic Mobility Rat Plasma t_0 with 2.0 mM PCMPS	27.0 ± 0.3	21.3 ± 0.1	16.2 ± 0.2[c]	13.2 ± 0.2[c]
Electrophoretic Mobility Rat Plasma t_4 with 2.0 mM PCMPS	26.4 ± 0.3	21.0 ± 0.3	16.0 ± 0.2[c]	13.9 ± 0.3[b,c]
% Change in Electrophoretic Mobility t_4 compared to Rat Plasma t_0 with 2.0 mM PCMPS	-2.2%	-1.4%	-1.3%	5.3%

[a] Results are expressed as the mean ± S.E.M. of 9 animals
[b] Significantly different from Rat Plasma t_0 value $p < 0.05$
[c] Significantly different from Rat Plasma t_4 value $p < 0.05$

APPENDIX A

RAT SERUM STORAGE AND THE LIPOPROTEIN-CHOLESTEROL PROFILE

TABLE 5

STORAGE EFFECTS ON THE LIPOPROTEIN-CHOLESTEROL CONCENTRATION OF RAT SERUM

	FRESH SERUM	SERUM STORED 4 °C, 4 HOURS	SERUM STORED -20 °C, 4 HOURS	SERUM STORED 4 °C, 24 HOURS	SERUM STORED -20 °C, 24 HOURS
PAL-C	9.2 ± 2.2	12.1 ± 3.1	11.1 ± 2.9	12.7 ± 3.8	12.0 ± 3.9
α_1L-C	21.5 ± 2.7	18.5 ± 2.3	19.4 ± 2.3	19.4 ± 2.3	21.0 ± 2.2
α_2L-C	4.1 ± 0.4	4.6 ± 0.5	4.0 ± 0.3	4.0 ± 0.8	3.1 ± 0.4
βL-C	10.8 ± 1.2	10.3 ± 0.9	11.0 ± 1.3	9.4 ± 0.9	9.3 ± 0.5

Values represent the mean cholesterol concentration in mg/dl ± S.E.M. of 4 animals fasted overnight

Aliquots of rat serum were analyzed for lipoprotein-cholesterol content by cellulose acetate electrophoresis combined with an enzymatic stain for total cholesterol immediately after serum collection, after 4 hours at 4 °C, after 4 hours at -20 °C, after 24 hours at 4 °C or after 24 hours at -20 °C. Results are shown in table 5 above. None of the lipoprotein fractions was found to be statistically different under any of the five conditions as determined by analysis of variance. Therefore, the effects of serum storage on the observed phenomena as reported in this dissertation are minor and would not necessarily affect the conclusions drawn from these studies.

APPENDIX B

TABLE 6

EFFECTS OF A BACTERIOSTATIC AGENT ON THE LIPOPROTEIN-CHOLESTEROL CONCENTRATION OF ADULT MALE RAT PLASMA AS DETERMINED BY CELLULOSE ACETATE ELECTROPHORESIS BEFORE AND AFTER A FOUR HOUR 37 °C INCUBATION

	RELATIVE % LIPOPROTEIN-CHOLESTEROL PRIOR TO INCUBATION		RELATIVE % LIPOPROTEIN CHOLESTEROL AFTER A 4 HOUR INCUBATION AT 37 °C	
	CONTROL PLASMA	PLASMA CONTAINING 0.015% THIMEROSAL	CONTROL PLASMA	PLASMA CONTAINING 0.015% THIMEROSAL
PAL-C	29.0 ± 3.3	29.6 ± 4.3	69.1 ± 3.6[a]	76.1 ± 3.7[a]
α_1L-C	47.2 ± 1.7	45.8 ± 3.1	19.5 ± 2.5[a]	13.5 ± 2.6[a]
α_2L-C	5.6 ± 0.5	5.4 ± 0.9	2.1 ± 0.2[a]	2.0 ± 0.1[a]
βL-C	18.2 ± 1.6	19.2 ± 1.6	9.3 ± 1.4[a]	8.4 ± 1.5[a]

Values represent the mean ± S.E.M. of 4 animals fasted overnight.

[a] Significantly different vs. non-incubated plasma using Duncan's new multiple range test ($p < 0.05$)

A bacteriostatic agent, thimerosal, was added to plasma samples at a final concentration of 0.015%. No significant differences were observed between non-incubated plasma with and without thimerosal or between plasma incubated at 37 °C for 4 hours with and without thimerosal. Apparent movement of cholesterol from α_1L to PAL was observed in plasma incubated for 4 hours at 37 °C whether thimerosal was present or not as indicated by table 6 above. In addition, a similar transfer of cholesterol from α_1L to PAL was observed under sterile techniques. This indicates that the movement of cholesterol from α_1L-C to PAL-C was not due to bacterial contamination.

CHAPTER IX

BIBLIOGRAPHY

Acevedo, Hernan F., Campbell, Elizabeth A., Saier, Eleanor L., Frich, John C. Jr., Merkow, Leonard P., Hayeslip, David W., Bartok, Stephen P., Grauer, Robert C. and Hamilton, John L., Urinary cholesterol. V. Its excretion in men with testicular and prostatic neoplasms. Cancer 32: 196-205, 1973.

Alaupovic, P., The concepts, classification systems and nomenclatures of human plasma lipoproteins. In CRC Handbook of Electrophoresis Volume I Lena A. Lewis and Jan J. Opplt eds., CRC Press Inc. Boca Raton, FL, 1980, pp. 27-46.

Albers, John J., Wahl, Patricia W., Cabana, Veneracion G., Hazzard, William R. and Hoover, J. Joanne, Quantitation of apolipoprotein A-I of human plasma high density lipoprotein. Metabolism 25: 633-644, 1976.

Alexander, Cheryl A., Hamilton, Robert L. and Havel, Richard J., Subcellular localization of B apoprotein of plasma lipoproteins in the rat. J. Cell Biol. 69: 241-263, 1976.

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. and Fu, P. C., Enzymatic determination of total serum cholesterol. Clin. Chem. 20: 470-473, 1974.

Andersen, John M. and Dietschy, John M., Regulation of sterol synthesis in adrenal gland of the rat by both high and low density human plasma lipoprotein. Biochem. Biophys. Res. Comm. 72: 880-885, 1976.

Andersen, John M. and Dietschy, John M., Regulation of sterol synthesis in 15 tissues of rat. II. Role of rat and human high and low density plasma lipoproteins and of rat chylomicron remnants. J. Biol. Chem. 252: 3652-3659, 1977.

Andersen, John M. and Dietschy, John M., Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary and testis of the rat. J. Biol. Chem. 253: 9024-9032, 1978.

Andersen, John M. and Dietschy, John M., Absolute rates of cholesterol synthesis in extrahepatic tissues measured with ^{3}H-labeled water and ^{14}C-labeled substrates. J. Lipid Res. 20: 740-752, 1979.

Anderson, Richard G. W., Brown, Michael S. and Goldstein, Joseph L., Role of the coated endocytic vesicle in the uptake of receptor-bound low density lipoprotein in human fibroblasts. Cell 10: 351-364, 1977a.

Anderson, Richard G. W., Goldstein, Joseph L. and Brown Michael S., Localization of low density lipoprotein receptors on plasma membrane of normal human fibroblasts and their absence in cells from a familial hypercholesterolemia homozygote. Proc. Natl. Acad. Sci. 73: 2434-2438, 1976.

Anderson, Richard W., Goldstein, Joseph L. and Brown, Michael S., A mutation that impairs the ability of lipoprotein receptors to localize in coated pits on the cell surface of human fibroblasts. Nature 270: 695-699, 1977b.

Armstrong, S. H. Jr., Budka, M. J. E. and Morrison, K. C., Preparation and properties of serum and plasma proteins. XI. Quantitative interpretation of electrophoretic schlieren diagrams of normal human plasma proteins. J. Am. Chem. Soc. 69: 416-529, 1947.

Arntzenius, A. C., Stegerhoek, C. I. and Styblo, K., Reduced high-density lipoprotein in women aged 40-41 using oral contraceptives. Lancet 1: 1221-1223, 1978.

Aron, Lieselotte, Jones, Stephen and Fielding, Christopher, Human plasma lecithin-cholesterol acyltransferase characterization of cofactor dependent phospholipase activity. J. Biol. Chem. 253: 7220-7226, 1978.

Assman, Gerd, Brown, Greg B. and Mahley, Robert W., Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured swine aortic smooth muscle cells by plasma lipoproteins. Biochemistry 14: 3996-4002, 1975.

Baker, Derek P., Van Lenten, Brian J., Fogelman, Alan M., Edwards, Peter A., Kean, Charles and Berliner, Judith A., LDL, scavenger, and β-VLDL receptors on aortic endothelial cells. Arteriosclerosis 4: 248-255, 1984.

Balasubramaniam, S., Goldstein, Joseph L., Faust, Jerry R., Brunschede, Gloria Y. and Brown, Michael S., Lipoprotein-mediated regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesteryl ester metabolism in the adrenal gland of the rat. J. Biol. Chem. 252: 1771-1779, 1977.

Barr, David P., Influence of sex and sex hormones upon the development of atherosclerosis and upon the lipoproteins of plasma. J. Chronic Dis. 1: 63-85, 1955.

Barr, David P., Russ, Ella M. and Eder, Howard A., Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. Am. J. Med. 11: 480-493, 1951.

Barter, Phillip J., Origin of esterified cholesterol transported in the very low density lipoproteins of human plasma. J. Lipid Res. 15: 11-19, 1974a.

Barter, Phillip J., Production of plasma esterified cholesterol in lean, normotriglyceridemic humans. J. Lipid Res. 15: 234-242, 1974b.

Barter, Phillip J., Evidence that lecithin:cholesterol acyltransferase acts on both high-density and low-density lipoproteins. Biochim. Biophys. Acta 751: 261-270, 1983.

Barter, Phillip J. and Conner, William E., The transport of esterified cholesterol in plasma high density lipoproteins of human subjects: A mathematical model. J. Lab. Clin. Med. 88: 627-639, 1976.

Barter, P. J., Gooden, J. M. and Rajaram, O. V., Species differences in the activity of a serum triglyceride transferring factor. Atherosclerosis 33: 165-169, 1979a.

Barter, Phillip J., Gorjatschko, Ludmila and Calvert, G. Dennis, Net mass transfer of esterified cholesterol from human low density lipoproteins to very low density lipoproteins incubated in vitro. Biochim. Biophys. Acta 619: 436-439, 1980.

Barter, Phillip J., Hopkins, Garry J. and Calvert, G. Dennis, Pathways for the incorporation of esterified cholesterol into very low density and low density lipoproteins in plasma incubated in vitro. Biochim. Biophys. Acta 713: 136-148, 1982a.

Barter, Phillip J., Hopkins, Garry J., Gorjatschko, Ludmila and Jones, Michael E., A unified model of esterified cholesterol exchanges between human plasma lipoproteins. Atherosclerosis 44: 27-40, 1982b.

Barter, Phillip J., Hopkins, Garry J., Gorjatschko, Ludmila and Jones, Michael E. Competitive inhibition of plasma cholesterol esterification by human high-density lipoprotein-subfraction 2. Biochim. Biophys. Acta. 793: 260-269, 1984.

Barter, P. J. and Jones, M. E., Rate of exchange of esterified cholesterol between human plasma low and high density lipoproteins. Atherosclerosis 34: 67-74, 1979b.

Barter, P. J. and Lally, J. I., The activity of an esterified cholesterol transferring factor in human and rat serum. Biochim. Biophys. Acta 531: 233-236, 1978.

Barter, Phillip J. and Lally, Janice I., In vitro exchanges of esterified cholesterol between serum lipoprotein fractions: Studies of humans and rabbits. Metabolism 28: 230-236, 1979c.

Barter, Phillip J., Lally, Janice I. and Wattchow, David, Metabolism of triglyceride in rabbit plasma low and high density lipoproteins: Studies in vivo and in vitro. Metabolism 28: 614-618, 1979d.

Basu, Sandip K., Brown, Michael S., Ho, Y. K. and Goldstein, Joseph L., Degradation of low density lipoprotein-dextran sulfate complexes associated with deposition of cholesteryl esters in mouse macrophages. J. Biol. Chem. 254: 7141-7146, 1979.

Beckering, Raymond E. and Ellefson, Ralph D., A rapid method for lipoprotein electrophoresis using cellulose acetate as support medium. Am. J. Clin. Pathol. 53: 84-88, 1970.

Beher, W. T., Baker, G. D. and Penney, D. G., Comparative study of the effects of bile acids and cholesterol on cholesterol metabolism in the mouse, rat, hamster and guinea pig. J. Nutr. 79: 523-530, 1963.

Beisegel, Ulrike and Utermann, Gerd, An apolipoprotein homolog of rat apolipoprotein A-IV in human plasma. Eur. J. Biochem. 93: 601-608, 1979.

Benditt, Earl P. and Benditt, John M., Evidence for a monoclonal origin of human atherosclerotic plaques. Proc. Natl. Acad. Sci. 70: 1753-1756, 1973.

Benesch, Reinhold and Benesch, Ruth E., Determination of -SH groups in proteins. In Methods of Biochemical Analysis Vol. 10. David Glick ed., Interscience Publishers, New York, NY, 1962, pp. 43-70.

Bennion, L. J. and Grundy, S. M., Effects of obesity and caloric intake on biliary lipid metabolism in man. J. Clin. Invest. 56: 996-1011, 1975.

Bentzen, Craig L., Acuff, Kenneth J., Marechal, Bilkis, Rosenthal, Murray A. and Volk, Murray E., Direct determination of lipoprotein distribution with micro-scale affinity chromatography columns. Clin. Chem. 28: 1451-1456, 1982.

Bergman, E. N., Havel, R. J., Wolfe, B. M. and Bohmer, T., Quantitative studies of the metabolism of chylomicron triglycerides and cholesterol by liver and extrahepatic tissues of sheep and dogs. J. Clin. Invest. 50: 1831-1839, 1971.

Beutler, H. O. and Michal, G., Eine eigehaltsbestimmung fur die routineanalytik: Enzymatische bestimmung von cholesterin. Getreide Mehl Brot. 30: 116-118, 1976.

Bilheimer, David W., Eisenberg, Shlomo and Levy, Robert I., The metabolism of very low density lipoprotein proteins. I. Preliminary *in vitro* and *in vivo* observations. Biochim. Biophys. Acta. 260: 212-221, 1972.

Bilheimer, David W., Goldstein, Joseph L., Grundy, Scott M., Starzl, Thomas E. and Brown, Michael S., Liver transplantation to provide low-density-lipoprotein receptors and lower plasma cholesterol in a child with homozygous familial hypercholesterolemia. N. Engl. J. Med. 311: 1658-1664, 1984.

Bishop, Sanford P., Cardiovascular research, In The Laboratory Rat Vol. II. Research Applications. Henry J. Baker, J. Russell Lindsey and Steven H. Weisbroth eds., Academic Press, New York, NY, 1980, pp. 162-179.

Blackburn, Henry, The meaning of a new marker for coronary artery disease. N. Engl. J. Med. 309: 426-427, 1983.

Bloch, Konrad, The biological synthesis of cholesterol. Science 150: 19-28, 1965.

Bon, G. Bittolo, Cazzolato, G. and Avogaro, P., Preparative isotachophoresis of human plasma high density lipoproteins HDL_2 and HDL_3. J. Lipid Res. 22: 998-1002, 1981.

Bottiger, L. E., Boman, G., Eklund, G. and Westerholm, B., Oral contraceptives and thromboembolic disease: Effects of lowering oestrogen content. Lancet 1: 1097-1101, 1980a.

Bottiger, Lars-Erik and Carlson, Lars A., Risk factors for ischaemic vascular death for men in the Stockholm prospective study. Atherosclerosis 36: 389-408, 1980b.

Bradley, Douglas D., Wingerd, John, Petitti, Diana B., Krauss, Ronald M. and Ramcharan, Savitri, Serum high-density-lipoprotein cholesterol in women using oral contraceptives, estrogens and progestins. N. Engl. J. Med. 299: 17-20, 1978.

Bradley, W. A., Rohde, M. F. and Gotto, A. M. Jr., Studies on the primary structure of apolipoprotein B. Ann. N. Y. Acad. Sci. 348: 87-103, 1980.

Bragdon, Joseph H., Havel, Richard J. and Boyle, Edwin, Human serum lipoproteins I. Chemical composition of four fractions. J. Lab. Clin. Med. 48: 36-42, 1956.

Bratzler, Robert L., Chisolm, Guy M., Colton, Clark K., Smith, Kenneth A., Zilversmit, Donald B. and Lees, Robert S., The distribution of labeled albumin across the rabbit thoracic aorta in vivo. Circ. Res. 40: 182-190, 1977.

Briere, R. O. and Mull, J. D., Electrophoresis of serum protein with cellulose acetate a method for quantitation. Am. J. Clin. Pathol. 42: 547-551, 1964.

Brown, Michael S., Basu, Sandip K., Falck, J. R., Ho, Y. K. and Goldstein, Joseph L., The scavenger cell pathway for lipoprotein degradation: Specificity of the binding site that mediates the uptake of negatively-charged LDL by macrophages. J. Supra. Struct. 13: 67-81, 1980.

Brown, Michael S., Dana, Suzanna E. and Goldstein, Joseph L., Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins. Proc. Natl. Acad. Sci. 70: 2162-2166, 1973.

Brown, Michael S., Dana, Suzanna E. and Goldstein, Joseph L., Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured human fibroblasts. J. Biol. Chem. 249: 789-796, 1974a.

Brown, Michael S., Dana, Suzanna E. and Goldstein, Joseph L., Cholesterol ester formation in cultured human fibroblasts. Stimulation by oxygenated sterols. J. Biol. Chem., 250: 4025-4027, 1975a.

Brown, Michael S., Dana, Suzanna E. and Goldstein, Joseph L., Receptor-dependent hydrolysis of cholesteryl esters contained in plasma low density lipoprotein. Proc. Natl. Acad. Sci. 72: 2925-2929, 1975b.

Brown, Michael S., Faust, Jerry R. and Goldstein, Joseph L., Role of the low density lipoprotein receptor in regulating the content of free and esterified cholesterol in human fibroblasts. J. Clin. Invest. 55: 783-793, 1975c.

Brown, Michael S. and Goldstein, Joseph L., Familial hypercholesterolemia: Defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Proc. Natl. Acad. Sci. 71: 788-792, 1974b.

Brown, Michael S. and Goldstein, Joseph L., Regulation of activity of the low density lipoprotein receptor in human fibroblasts. Cell 6: 307-316, 1975d.

Brown, Michael S., Kovanen, Petri T. and Goldstein, Joseph L., Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex. Recent Prog. Horm. Res. 35: 215-257, 1979.

Brown, Michael S., Kovanen, Petri and Goldstein, Joseph L., Regulation of plasma cholesterol by lipoprotein receptors. Science 212: 628-635, 1981.

Bugugnani, M. J., Koffigan, M., Kora, I., Ouvry, D., Clavey, V. and Fruchart, J. C., Rapid assessment of the distribution of apolipoproteins C-II and C-III subspecies in triglyceride-rich lipoproteins by isoelectric focusing. Clin. Chem. 30: 349-351, 1984.

Buja, L. Maximilian, Kovanen, Petri T. and Bilheimer, David W., Cellular pathology of homozygous familial hypercholesterolemia. Am. J. Pathol. 97: 327-358, 1979.

Burstein, M. and Samaille, J., Sur un dosage rapide du cholesterol lie aux α et aux β-lipoproteins du serum. Clin. Chim. Acta 5: 609, 1960.

Burstein, M. and Scholnick, H. R., Lipoprotein-polyanion-metal interactions. Adv. Lipid Res. 11: 67-108, 1973.

Burstein, M., Scholnick, H. R. and Morfin, R., Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res. 11: 583-595, 1970.

Camejo, German, Bosch, Virgilio, Arreaza, Carlos and Mendez, Halina C., Early changes in plasma lipoprotein structure and biosynthesis in cholesterol-fed rabbits. J. Lipid Res. 14: 61-68, 1973.

Castelli, William P., Doyle, Joseph T., Gordon, Tavia, Hames, Curtis G., Hjortland, Marthana C., Hulley, Stephen B., Kagan, Abraham and Zukel, William J., HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation 55: 767-772, 1977.

Cauley, Jane A., LaPorte, Ronald E., Kuller, Lewis H., Bates, Margaret and Sandler, Rivka Black, Menopausal estrogen use, high density lipoprotein cholesterol subfractions and liver function. Atherosclerosis 49: 31-39, 1983.

Chait, Alan, Bierman, Edwin L. and Albers, John J., Regulatory role of triiodothyronine in the degradation of low density lipoprotein by cultured human skin fibroblasts. J. Clin. Endocrinol. Metab. 48: 887-889, 1979.

Chajek, Tova, Aron, Lieselotte and Fielding, Christopher J., Interaction of lecithin:cholesterol acyltransferase and cholesterol transfer protein in the transport of cholesterol ester into sphingomyelin liposomes. Biochemistry 19: 3673-3677, 1980.

Chajek, T. and Fielding, C. J., Isolation and characterization of a human serum cholesteryl ester transfer protein. Proc. Natl. Acad. Sci. 75: 3445-3449, 1978.

Chalvardjian, A., Agarose-starch gel electrophoresis of rat serum lipoproteins. J. Lipid Res. 12: 265-269, 1971.

Chao, Yu-sheng, Windler, Eberhard E., Chen, G. Chi and Havel, Richard J., Hepatic catabolism of rat and human lipoproteins in rats treated with 17 α-ethinyl estradiol. J. Biol. Chem. 254: 11360-11366, 1979.

Chapman, John M., Animal lipoproteins: Chemistry, structure and comparative aspects. J. Lipid Res. 21: 789-853, 1980.

Chen, Yii-Der I., Kraemer, Fredric B. and Reaven, Gerald M., identification of specific high density lipoprotein-binding sites in rat testis and regulation of binding by human chorionic gonadotropin. J. Biol. Chem. 255: 9162-9167, 1980.

Cheung, Marian C. and Albers, John J., The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. J. Clin. Invest. 60: 43-50, 1977.

Chin, H. P. and Blankenhorn, David H., Separation and quantitative analysis of serum lipoproteins by means of electrophoresis on cellulose acetate. Clin. Chim. Acta 20: 305-314, 1968.

Chong, Kui Song, Jahani, Mehrnoosh, Hara, Shinichi and Lacko, Andras G., Characterization of lecithin-cholesterol acyltransferase from human plasma 3. Chemical properties of the enzyme. Can. J. Biochem. Cell Biol. 61: 875-881, 1983.

Chow, S. L., Hollander, D., Initial cholesterol uptake by everted sacs of rat small intestine: kinetic and thermodynamic aspects. Lipids 13: 239-245, 1978.

Clarenburg, R. and Chaikoff, I. L., Origin of milk cholesterol in the rat: Dietary versus endogenous sources. J. Lipid Res. 7: 27-37, 1966.

Clarkson, Priscilla M., Hintermister, Robert, Fillyaw, Micahel and Stylos, Louis, High density lipoprotein cholesterol in young adult weight lifters, runners and untrained subjects. Hu. Bio. 53: 251-257, 1981.

Cobb, Sharon A. and Sanders, James L., Enzymic determination of cholesterol in serum lipoproteins separated by electrophoresis. Clin. Chem. 24: 1116-1120, 1978.

Coleman, Rosalind and Bell, Robert M., Evidence that biosynthesis of phosphatidylethanolamine, phosphatidylcholine and triacylglycerol occurs on the cytoplasmic side of microsomal vessicles. J. Cell Biol. 76: 245-253, 1978.

Conlon, Dennis R., Blankstein, Larry A., Pasakarnis, Pamela A., Steinberg, Carolyn M. and D'Amelio, Jane E., Quantitative determination of high-density lipoprotein cholesterol by agarose gel electrophoresis. Clin. Chem. 25: 1965-1969, 1979.

Cook, R. P. and Thompson, R. O., The absorption of fat and of cholesterol in the rat guinea-pig and rabbit. Quart. J. Exp. Physiol. 36: 61-74, 1951.

Cookson, F. B., The origin of foam cells in atherosclerosis. Br. J. Exp. Pathol. 52: 62-69, 1971.

Crouse, John R. III, Progress in coronary artery disease risk-factor research: What remains to be done? Clin. Chem. 30: 1125-1127, 1984.

Curry, Michael D., Gustafson, Anders, Alaupovic, Petar and McConathy, Walter J., Electroimmunoassay, radioimmunoassay and radial immunodiffusion assay evaluated for quantification of human apolipoprotein B. Clin. Chem. 24: 280-286, 1978.

Daerr, Wolfgang H., Windler, Eberhard E. T., Rohwer, Hans-Dietrich and Greten, Heiner, Limitations of a new double-precipitation method for the determination of high density lipoprotein subfractions 2 and 3. Atherosclerosis 49: 211-213, 1983.

Damen, Jan, Regts, Joke and Scherphof, Gerrit, Transfer of (^{14}C) phosphatidylcholine between liposomes and human plasma high density lipoprotein. Partial purification of a transfer-stimulating plasma factor using a rapid transfer assay. Biochim. Biophys. Acta 712: 444-452, 1982.

Davis, Roger A. and Roheim, Paul S. Pharmacologically induced hypolipidemia. Atherosclerosis 30: 293-299, 1978.

DeLamatre, John G. and Roheim, Paul S., Effect of cholesterol feeding on apo B and apo E concentrations and distributions in euthyroid and hypothyroid rats. J. Lipid Res. 22: 297-306, 1981.

Dietschy, John M. and Gamel, W. G., Cholesterol synthesis in the intestine of man: Regional differences and control mechanisms. J. Clin. Invest. 50: 872-880, 1971.

Dietschy, J. M. and Siperstein, M. D., Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. J. Lipid Res. 8: 97-104, 1967.

Dory, Ladislav and Roheim, Paul S., Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism. J. Lipid Res. 22: 287-296, 1981.

Drevon, Christian A., Attie, Alan D., Pangburn, Sharon H. and Steinberg, Daniel, Metabolism of homologous and heterologous lipoproteins by cultured rat and human skin fibroblasts. J. Lipid Res. 22: 37-46, 1981.

Dyer, Alan R., Stamler, Jerimiah, Paul, Oglesby, Lepper, Mark, Shekelle, Richard B., McKean, Harlley and Garside, Dan, Alcohol consumption and 17-year mortality in the Chicago Western Electric Company study. Prev. Med. 9: 78-90, 1980.

Eggena, Peter, Tivol, William and Aladjem, Fredrick, Isoelectric heterogeneity of human serum high-density lipoproteins. Biochem. Med. 6: 184-188, 1972.

Eisenberg, Shlomo, Bilheimer, David W., Levy, Robert I. and Lindgren, Frank T., On the metabolic conversion of human plasma very low density lipoprotein to low density lipoprotein. Biochim. Biophys. Acta 326: 361-377, 1973a.

Eisenberg, Shlomo, Chajek, Tova and Deckelbaum, Richard J., The plasma origin of low density and high density lipoproteins. In Metabolic Risk Factors in Ischemic Cardiovascular Disease. L. A. Carlson and B. Pernow eds., Raven Press, New York, NY, 1982, pp. 59-67.

Eisenberg, Shlomo, Oschry, Yitzchak and Zimmerman, Joseph, Intravascular metabolism of the cholesteryl ester moiety of rat plasma lipoproteins. J. Lipid Res. 25: 121-128, 1984.

Eisenberg, Shlomo and Rachmilewitz, Daniel, Metabolism of rat plasma very low density lipoprotein I. Fate in circulation of the whole lipoprotein. Biochim. Biophys. Acta 326: 378-390, 1973b.

Eisenberg, Shlomo and Rachmilewitz, Daniel, Interaction of rat plasma very low density lipoprotein with lipoprotein lipase-rich (postheparin) plasma. J. Lipid Res. 16: 341-351, 1975.

Ellman, George L., Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82: 70-77, 1959.

Enholm, Christian, Shaw, Walter, Greten, Heiner and Brown, W. Virgil, Purification from human plasma of a heparin-released lipase with activity against triglyceride and phospholipids. J. Biol. Chem. 250: 6756-6761, 1975.

Faergeman, Ole, Sata, Tsunako, Kane, John P. and Havel Richard J., Metabolism of apoprotein B of plasma very low density lipoproteins in the rat. J. Clin. Invest. 56: 1396-1403, 1975.

Fainaru, M., Havel, R. J. and Imaizumi, K., Apoprotein content of plasma lipoproteins of the rat separated by gel chromatography or ultracentrifugation. Biochem. Med. 17: 347-355, 1977.

Farish, Elizabeth, Fletcher, Colin D., Hart, David M., Kitchener, Henry and Sharpe, Graham L. M., A long-term study of the effects of norethisterone on lipoprotein metabolism in menopausal women. Clin. Chim. Acta 132: 193-198, 1983.

Felker, Tunde, Fainaru Menahem, Hamilton, Robert L. and Havel, Richard J., Secretion of arginine-rich and A-I apolipoproteins by the isolated perfused rat liver. J. Lipid Res. 18: 465-473, 1977.

Fielding, C. J., Human lipoprotein lipase I. Purification and substrate specificity. Biochim. Biophys. Acta. 206: 109-117, 1970.

Fielding, C. J. and Fielding, P. E., Purification and substrate specificity of lecithin-cholesterol acyl transferase from human plasma. FEBS Lett. 15: 355-358, 1971.

Fielding, Christopher J. and Fielding, Phoebe E., Regulation of human plasma lecithin:cholesterol acyltransferase activity by lipoprotein acceptor cholesteryl ester content. J. Biol. Chem. 256: 2102-2104, 1981a.

Fielding, Christopher J. and Fielding, Phoebe E., Evidence for a lipoprotein carrier in human plasma catalyzing sterol efflux from cultured fibroblasts and its relationship to lecithin:cholesterol acyltransferase. Proc. Natl. Acad. Sci. 78: 3911-3914, 1981b.

Fielding, Phoebe and Fielding, Christopher J., A cholesterol ester transfer complex in human plasma. Proc. Natl. Acad. Sci. 77: 3327-3330, 1980.

Fielding, C. J., Havel, R. J., Lipoprotein lipase. Arch. Pathol. 101: 225-229, 1977.

Fielding, C. J., Shore, V. G. and Fielding, P. E., Lecithin:cholesterol acyltransferase: effects of substrate composition upon enzyme activity. Biochim. Biophys. Acta 270: 513-518, 1972a.

Fielding, C. J., Shore, V. G. and Fielding, P. E., A protein cofactor of lecithin:cholesterol acyltransferase. Biochem. Biophys. Res. Comm. 46: 1493-1498, 1972b.

Fillios, L. C., Andrus, S. B., Mann, G. V. and Stare, F. J., Experimental production of gross atherosclerosis in the rat. J. Exp. Med. 104: 539-554, 1956.

Fletcher, Maragaret J. and Styliou, Maria, A simple method for separating serum lipoproteins by electrophoresis on cellulose acetate. Clin. Chem. 16: 362-365, 1970.

Fogelman, Alan M., Haberland, Margaret E., Seager Janet, Hokom, Martha and Edwards, Peter A., Factors regulating the activities of the low density lipoprotein receptor and the scavenger receptor on human monocyte-macrophages. J. Lipid Res. 22: 1131-1141, 1981.

Forte, T., Norum, K. R., Glomset, J. A. and Nichols, A. V., Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: Structure of low and high density lipoproteins as revealed by electron microscopy. J. Clin. Invest. 50: 1141-1148, 1971.

Frantz, Ivan D. Jr., Schneider, Henny S. and Hinkelman, Beverly T., Suppression of hepatic cholesterol synthesis in the rat by cholesterol feeding. J. Biol. Chem. 206: 465-469, 1954.

Fredrickson, D. S. and Lees, R. S., A system for phenotyping hyperlipoproteinemia. Circ. 31: 321-327, 1965.

Frnka, Jerome and Reiser, Raymond, The effects of diet cholesterol on the synthesis of rat serum apolipoproteins. Biochim. Biophys. Acta 360: 322-338, 1974.

Frolich, J., Bernstein, Victoria and Bernstein, M., Lecithin:cholesterol acyltransferase. Initial fractional rates of esterification in human and rat serum during development. Clin. Chim. Acta 65: 79-82, 1975.

Gallo, L. L., Treadwell, C. R., Localization of cholesterol esterase and cholesterol in mucosal fractions of rat small intestine. Proc. Soc. Exp. Biol. Med. 114: 69-72, 1963.

Gidez, Lewis I., Miller, George J., Burstein, Meier, Slagle, Susan and Eder, Howard, Separation and quantitation of subclassed of human plasma high density lipoproteins by a simple precipitation procedure. J. Lipid Res. 23: 1206-1223, 1982.

Gidez, Lewis I., Roheim, Paul S. and Eder, Howard A., Turnover of cholesteryl esters of plasma lipoproteins in the rat. J. Lipid Res. 8: 7-15, 1967.

Glomset, John A., The mechanism of the plasma cholesterol esterification reaction: Plasma fatty acid transferase. Biochim. Biophys. Acta 65: 128-135, 1962.

Glomset, John A., Physiological role of lecithin-cholesterol acyltransferase. Am. J. Clin. Nutr. 23: 1129-1136, 1970.

Glomset, J. A., Janssen, E. T., Kennedy, R. and Dobbins, J., Role of plasma lecithin:cholesterol acyltransferase in the metabolism of high density lipoproteins. J. Lipid Res. 7: 638-648, 1966.

Glomset, John A. and Norum, Kaare R., The metabolic role of lecithin:cholesterol acyltransferase: Perspectives from pathology. Adv. Lipid Res. 11: 1-63, 1973.

Glomset, John A., Norum, Kaare R. and King, Weiling, Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: Lipid composition and reactivity in vitro. J. Clin. Invest. 49: 1827-1837, 1970.

Godolphin, W. J. and Stinson, R. A., Isoelectricfocusing of human plasma lipoproteins in polyacrylamide gels: Diagnosis of type III hyperlipoproteinemia ("broad beta" disease). Clin. Chim. Acta 56: 97-103, 1974.

Gofman, John W., Lindgren, Frank T. and Elliott, Harold, Ultracentrifugal studies of lipoproteins of human serum. J. Biol. Chem. 179: 973-979, 1949.

Gofman, John W., Rubin, Leonard, McGinley, James P. and Jones, Hardin B., Hyperlipoproteinemia. Am. J. Med. 17: 514-520, 1954.

Goldstein, Joseph L., Basu, Sandip K., Brunschede, Gloria Y. and Brown, Michael S., Release of low density lipoprotein from its cell surface receptor by sulfated glycosaminoglycans. Cell 7: 85-95, 1976.

Goldstein, Joseph L. and Brown, Michael S., Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. J. Biol. Chem. 249: 5153-5162, 1974a.

Goldstein, Joseph L. and Brown, Michael S., The low-density lipoprotein pathway and its relation to atherosclerosis. Ann. Rev. Biochem. 46: 897-930, 1977a.

Goldstein, Joseph L. and Brown, Michael S., Atherosclerosis: The low-density lipoprotein receptor hypothesis. Metabolism 26: 1257-1275, 1977b.

Goldstein, Joseph L., Brunschede, Gloria Y. and Brown, Michael S., Inhibition of the proteolytic degradation of low density lipoprotein in human fibroblasts by chloroquine, concanavalin A, and triton WR 1339. J. Biol. Chem. 250: 7854-7862, 1975.

Goldstein, Joseph L, Dana, Suzanna E. and Brown, Michael S., Esterification of low density lipoprotein cholesterol in human fibroblasts and its absence in homozygous familial hypercholesterolemia. Proc. Natl. Acad. Sci. 71: 4288-4292, 1974b.

Goldstein, Joseph L., Ho, Y. K., Basu, Sandip K. and Brown, Michael S., Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein producing massive cholesterol deposition. Proc. Natl. Acad. Sci. 76: 333-337, 1979.

Goodman, DeWitt S., The metabolism of chylomicron cholesterol ester in the rat. J. Clin. Invest. 41: 1886-1896, 1962.

Goodman, DeWitt S., The *in vivo* turnover of individual cholesterol esters in human plasma lipoproteins. J. Clin. Invest. 43: 2026-2036, 1964.

Gordon, Tavia, Castelli, William P., Hjortland, Marthana C., Kannel, William B. and Dawber, Thomas R., High density lipoprotein as a protective factor against coronary heart disease, The Framingham study. Am. J. Med. 62: 707-714, 1977.

Green, Peter H. R., Glickman, Robert M., Sandek, Christopher D., Blum, Conrad B. and Tall, Alan R., Human intestinal lipoproteins: studies in chyluric subjects. J. Clin. Invest. 64: 233-242, 1979.

Green, P. H. R., Tall, A. R. and Glickman, R. M., Rat intestine secretes discoid high density lipoprotein. J. Clin. Invest. 61: 528-534, 1978.

Grice, H. C., Methods for obtaining blood and intravenous injections in laboratory animals. Lab Animal Care 14: 483-493, 1964.

Groener, J. E. M., Van Rozen, A. J. and Erkelens, D. W., Cholesteryl ester transfer activity, localization and role in distribution of cholesteryl ester among lipoproteins in man. Atherosclerosis 50: 261-271, 1984.

Grundy, Scott M., Cholesterol metabolism in man. West. J. Med. 128: 13-25, 1978.

Grundy, Scott M., Atherosclerosis: Pathology, pathogenesis and role of risk factors. DM 29: 1-58, 1983.

Grundy, Scott M. and Metzger, S. L., A physiological method for estimation of hepatic secretion of biliary lipids. Gastroenterology 62: 1200-1217, 1972.

Grundy, Scott M. and Mok, Henry Y. I., Chylomicron clearance in normal and hyperlipidemic man. Metabolism 25: 1225-1239, 1976.

Gwynne, John T. and Hess, Barbara, The role of high density lipoproteins in rat adrenal cholesterol metabolism and steroidogenesis. J. Biol. Chem. 255: 10875-10883, 1980.

Gwynne, J. T., Mahaffee, D., Brewer, H. B. Jr. and Ney, R. L., Adrenal cholesterol uptake from plasma lipoproteins. Regulation by corticotropin. Proc. Natl. Acad. Sci. 73: 4329-4333, 1976.

Ha, Ying Chea and Barter, Phillip J., Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. Comp. Biochem. Physiol. 71B: 265-269, 1982.

Hamilton, L. D. G., The estimation of side chain groups in the intact protein. In A Laboratory Maunal of Analytical Methods of Protein Chemistry Including Polypeptides. P. Alexander and R. J. Block eds., Pergamon Press, New York, NY, 1960, pp. 59-100.

Hamilton, Robert L., Synthesis and secretion of plasma lipoproteins. In Pharmacological Control of Lipid Metabolism. W. I. Holmes, R. Paoletti and D. Kritchevsky eds., Plenum Press, New York, NY, 1972, pp.7-24.

Hamilton, Robert L., Nascent VLDL and nascent HDL from liver. In Proc. 5th Int. Symp. Atherosclerosis. A. M. Gotto, L. C. Smith and B. Allen eds., Springer Verlag, New York, NY, 1980, pp. 164-167.

Hamilton, Robert L., Regen, David M., Gray, Mary E. and Lequire, V. S., Lipid transport in liver I. Electron microscopic identification of very low density lipoproteins in perfused rat liver. Lab. Invest. 16: 305-319, 1967.

Hamilton, Robert L., Williams, Mary C., Fielding, Christopher J. and Havel, Richard J., Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. J. Clin. Invest. 58: 667-680, 1976.

Hardison, William G. M. and Apter, Julia T., Micellar theory of biliary cholesterol excretion. Am. J. Physiol. 222: 61-67, 1972.

Hare, David L., Stimpson, Donald I. and Cann, John R., Multiple bands produced by interaction of a single macromolecule with carrier ampholytes during isoelectric focusing. Arch. Biochem. Biophys. 187: 274-275, 1978.

Hartung, G. Harley, Foreyt, John P., Mitchell, Robert E., Vlasek, Imogene and Gotto, Antonio M., Relation of diet to high-density-lipoprotein cholesterol in middle-aged marathon runners, joggers and inactive men. N. Engl. J. Med. 302: 357-361, 1980.

Hashimoto, Sam and Fogelman, Alan M., Smooth microsomes. A trap for cholesteryl ester formed in hepatic microsomes. J. Biol. Chem. 255: 8678-8684, 1980.

Haskell, William L., Camargo, Carlos Jr., Williams, Paul T., Vranizan, Karen M., Krauss, Ronald M., Lindgren, Frank T. and Wood Peter D., The effect of cessation and resumption of moderate alcohol intake on serum high-density-lipoprotein subfractions. N. Engl. J. Med. 310: 805-810, 1984.

Hatch, Fredrick T. and Lees, Robert S., Practical methods for plasma lipoprotein analysis. Adv. Lipid Res. 6: 1-68, 1968.

Haugen, R. and Norum, K. R., Coenzyme A dependent esterification of cholesterol in rat intestinal mucosa. Scand. J. Gastroenterol. 11: 615-621, 1976.

Havel, Richard J., Lipoproteins and lipid transport, In Lipids, Lipoproteins and Drugs. David Kritchevsky, Rudolfo Paoletti and William L. Holmes, eds., Plenum Press, New York, NY, 1975, pp. 37-59.

Havel, R. S., Eder, H. A. and Bragdon, J. H., The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J. Clin. Invest. 34: 1345-1353, 1955.

Havel, Richard J., Fielding, Christopher J., Olivecrona, Thomas, Shore, Virgie G., Fielding, Phoebe E. and Egelrud, Torbjorn, Cofactor activity of protein components of human very low density lipoproteins in hydrolysis of triglycerides by lipoprotein lipase from different sources. Biochemistry 12: 1828-1833, 1973a.

Havel, Richard J., Goldstein, Joseph, L. and Brown, Michael S., Lipoproteins and lipid transport, In Metabolic Control and Disease 8th Edition. Phillip K. Bondy and Leon E. Rosenberg eds., W.B. Saunders Co., Philidelphia, PA, 1980, pp. 393-494.

Havel, Richard J. and Kane, John P., Primary dysbetalipoproteinemia: Predominance of a specific apoprotein species in triglyceride-rich lipoproteins. Proc. Natl. Acad. Sci. 70: 2015-2019, 1973b.

Havel, Richard J., Kane, John P. and Kashyap, Moti L., Interchange of apoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. J. Clin. Invest. 52: 32-38, 1973c.

Hay, R. V., Pottenger, L. A. Reingold, A. L., Getz, G. S. and Wissler, R. W., Degradation of I^{125}-labeled serum low density lipoprotein in normal and estrogen-treated male rats. Biochem. Biophys. Res. Comm. 44: 1471-1477, 1971.

Hayashi, Eiichi and Tomita, Takako, How does hypertension accelerate atherosclerosis? A possible mechanism from changes in aortic enzymes. TIPS 3: 31-38, 1982.

Heller, R. F., Jacobs, H. S., Vermeulen, A. and Deslypere, J. P., Androgens, oestrogens and coronary heart disease. Brit. Med. J. 282: 438-439, 1981a.

Heller, R. F., Miller, N. E., Lewis, B., Vermeulen, A., Fairney, Angela, James, V. H. T. and Swan, A. V., Association between sex hormones, thyroid hormones and lipoproteins. Clin. Sci. 61: 649-651, 1981b.

Hellerman, Leslie, Chinard, Francis P. and Dietz, Victor R., Protein sulfhydryl groups and the reversible inactivation of the enzyme urease. J. Biol. Chem. 147: 443-462, 1943.

Henriksen, Tore, Mahoney, Eileen M. and Steinberg, Daniel, Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: Recognition by receptors for acetylated low density lipoproteins. Proc. Natl. Acad. Sci. 78: 6499-6503, 1981.

Herbert, Peter N., Windmueller, Herbert G., Bersot, Thomas P. and Shulman, Richard S., Characterization of the rat apolipoproteins I. The low molecular weight proteins of rat plasma high density lipoproteins. J. Biol. Chem. 249: 5718-5724, 1974.

Ho, Y. K., Brown, Michael S. and Goldstein, Joseph L., Hydrolysis and excretion of cytoplasmic cholesteryl esters by macrophages: Stimulation by high density lipoprotein and other agents. J. Lipid Res. 21: 391-398, 1980.

Hoch, Hans and Chanutin, Alfred, An electrophoretic study of human plasma stored at room temperature. J. Biol. Chem. 209: 661-669, 1954.

Hofman, A. F. and Borgstrom, B., The intraluminal phase of fat digestion in man: The lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. J. Clin. Invest. 43: 247-257, 1964.

Hojnacki, Jerome L., Nicolosi, Robert J., Hoover, Gayle, Llansa, Norma, Ershow, Abby G., Lozy, Mohamed El and Hayes, K. C., Comparison of two ultracentrifugation procedures for separation of nonhuman primate lipoproteins. Anal. Biochem. 88: 485-494, 1978.

Hopkins, Garry J. and Barter, Phillip J., Transfers of esterified cholesterol and triglyceride between high density and very low density lipoproteins: In vitro studies of rabbits and humans. Metabolism 29: 546-550, 1980.

Hopkins, Garry J. and Barter, Phillip J., Dissociation of the in vitro transfers of esterified cholesterol and triglyceride between human lipoproteins. Metabolism 31: 78-81, 1982.

Houtsmuller, A. J., Heparin-induced post-β-lipoprotein. Lancet 2: 967-968, 1966.

Hulley, Stephen B., Rosenman, Ray H., Bawol, Richard D. and Brand, Richard J., Epidemiology as a guide to clinical decisions. The association between triglyceride and coronary heart disease. N. Engl. J. Med. 302: 1383-1389, 1980.

Hulsmann, W. C. and Jansen, H., High myocardial and low hepatic lipoprotein lipase activities responsible for the initiation of atherosclerosis. Biochem. Med. 13: 293-297, 1975.

Ihm, Jahei, Ellsworth, Jeff L., Chataing, Bernardo and Harmony, Judith A. K., Plasma protein-facilitated coupled exchange of phosphatidylcholine and cholesteryl ester in the absence of cholesterol esterification. J. Biol. Chem. 257: 4818-4827, 1982.

Ihm, Jahei and Harmony, Judith A. K., Simultaneous transfer of cholesteryl ester and phospholipid by protein(s) isolated from human lipoprotein-free plasma. Biochem. Biophys. Res. Comm. 93: 1114-1120, 1980.

Imaizumi, Katsumi, Fainaru, Menahem and Havel, Richard J., Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. J. Lipid Res. 19: 712-722, 1978a.

Imaizumi, Katsumi, Havel, Richard J., Fainaru, Menahem and Vigne, Jean-Louis, Origin and transport of the A-I and arginine-rich apolipoproteins in mesenteric lymph of rats. J. Lipid Res. 19: 1038-1046, 1978b.

Innerarity, Thomas L., Mahley, Robert W., Weisgraber, Karl H. and Bersot, Thomas P., Apoprotein (E-AII) complex of human plasma lipoproteins II. Receptor binding activity of a high density lipoprotein subfraction modulated by the Apo (E-AII) complex. J. Biol. Chem. 253: 6289-6295, 1978.

Innerarity, Thomas L., Pitas, Robert E. and Mahley, Robert W., Disparities in the interaction of rat and human lipoproteins with cultured rat fibroblasts and smooth muscle cells. Requirements for homology for receptor binding activity. J. Biol. Chem. 255: 11163-11172, 1980.

Jahani, Mehrnoosh and Lacko, Andras G., A study of the interaction of lecithin:cholesterol acyltransferase with subfractions of high density lipoproteins. J. Lipid Res. 22: 1102-1110, 1981.

Jansen, H. and Birkenhager, J. C., Liver lipase-like activity in human and hamster adrenocortical tissue. Metabolism 30: 428-430, 1981.

Jansen, Hans and Hulsmann, Willem C., Heparin-releasable (liver) lipase(s) may play a role in the uptake of cholesterol by steroid-secreting tissues. Trends Biochem. Sci. 5: 265-268, 1980.

Jansen, H., Schoonderwoerd, G. C. and Dallinga-Thie, G. M., Separation of rat plasma HDL subfractions by density gradient centrifugation and the effect of incubation on these fractions. Biochim. Biophys. Acta 754: 271-278, 1983.

Jenkins, P. J., Harper, R. W. and Nestel, P. J., Severity of coronary atherosclerosis related to lipoprotein concentration. Brit. Med. J. 2: 388-391, 1978.

Jeske, Debra J. and Dietschy, John M., Regulation of rates of cholesterol synthesis in vivo in the liver and the carcass of the rat measured using ^{3}H water. J. Lipid Res. 21: 364-376, 1980.

Johansson, Magnes B. and Karlsson, Borje W., Lipoproteins in serum of rat, mouse, gerbil, rabbit, pig and man studied by electrophoretical and immunological methods. Comp. Biochem. Physiol. 54B: 495-500, 1976.

Jonas, Ana, Interaction of bovine serum high density lipoprotein with mixed vesicles of phosphatidylcholine and cholesterol. J. Lipid Res. 20: 817-824, 1979.

Kashyap, M. L., Srivastava, L. S., Chen, C. Y., Perisutti, G., Campbell, M., Lutmer, R. F. and Glueck, C. J., Radioimmunoassay of human apolipoprotein CII. J. Clin. Invest. 60: 171-180, 1977.

Kirk, Roger E., Experimental Design: Procedures for the Behavioral Sciences Second Edition, Brooks/Cole Publishing Co., Monterey, CA, 1982.

Klatsky, Arthur L., Friedman, Gary D. and Siegelaub, Abraham B., Alcohol consumption before myocardial infarction: Results from the Kaiser-Permanente epidemiologic study of myocardial infarction. Ann. Int. Med. 81: 294-301, 1974.

Koga, Shunichi, Horwitz, David L. and Scanu, Angelo M., Isolation and properties of lipoproteins from normal serum. J. Lipid Res. 10: 577-588, 1969.

Kolata, Gina, New puzzles over estrogen and heart disease. Science 220: 1137-1138, 1983.

Kostner, Gerhard M., Enzymatic determination of cholesterol in high-density lipoprotein fractions prepared by polyanion precipitation. Clin. Chem. 22: 695, 1976.

Kostner, Gerhard M., The influence of various lipoproteins and apolipoproteins on the in vitro esterification of cholesterol in human serum by the enzyme lecithin:cholesterol acyltransferase. Scand. J. Clin. Invest. 38: 66-71, 1978.

Kovanen, Petri T., Basu, Sandip K., Goldstein, Joseph L. and Brown, Michael S., Low density lipoprotein receptors in bovine adrenal cortex. II. Low density lipoprotein binding to membranes prepared from fresh tissue. Endocrinology 104: 610-616, 1979a.

Kovanen, Petri T., Brown, Michael S. and Goldstein, Joseph L., Increased binding of low density lipoprotein to liver membranes from rats treated with 17 α-ethinyl estradiol. J. Biol. Chem. 254: 11367-11373, 1979b.

Kramsch, Dieter M., Aspen, Anita J. and Rozler, Lynn J., Atherosclerosis: Prevention by agents not affecting abnormal levels of blood lipids. Science 213: 1511-1512, 1981.

Krauss, Ronald M., Levy, Robert I. and Fredrickson, Donald S., Selective measurement of two lipase activities in post heparin plasma from normal subjects and patients with hyperlipoproteinemia. J. Clin. Invest. 54: 1107-1124, 1974.

Krauss, R. M., Windmueller, H. G., Levy, R. I. and Fredrickson, D. S., Selective measurement of two different triglyceride lipase activities in rat post heparin plasma. J. Lipid Res. 14: 286-295, 1973.

Kunitake, Steven T. and Kane, John P., Factors affecting the integrity of high density lipoproteins in the ultracentrifuge. J. Lipid Res. 23: 936-940, 1982.

Kuusi, Timo, Nikkila, Esko A., Virtanen, Ismo and Kinnunen, Paavo K. J., Localization of the heparin-releasable lipase in situ in the rat liver. Biochem. J. 181: 245-246, 1979.

Lally, Janice I. and Barter, Phillip J., The in vivo metabolism of esterified cholesterol in the plasma high-density lipoproteins of rabbits. J. Lab. Clin. Med. 93: 570-582, 1979.

Langer, T., Strober, W. and Levy, R. I., The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. J. Clin. Invest. 51: 1528-1536, 1978.

LaRosa, J. C., Levy, R. I., Hebert, P., Lux, S. E. and Fredrickson D. S., A specific apoprotein activator for lipoprotein lipase. Biochem. Biophys. Res. Comm. 41: 57-62, 1970.

Laskarzewski, P. M., Morrison, J. A., Gutai, J., Khoury, P. R. and Glueck, C. J., Longitudinal relationships among endogenous testosterone, estradiol and quetelet index with high and low density lipoprotein cholesterols in adolescent boys. Pediatr. Res. 17: 689-698, 1983.

Lasser, Norman L., Roheim, Paul S., Edelstein, Diane and Eder, Howard A., Serum lipoproteins of normal and cholesterol-fed rats. J. Lipid Res. 14: 1-8, 1973.

Lees, Robert S. and Fredrickson, Donald S., The differentiation of exogenous and endogenous hyperlipemia by electrophoresis. J. Clin. Invest. 44: 1968-1977, 1965.

Lees, Robert S. and Hatch, Fredrick T., Sharper separation of lipoprotein species by paper electrophoresis in albumin-containing buffer. J. Lab. Clin. Med. 61: 518-528, 1963.

Leeson, Thomas S. and Leeson, C. Roland, Histology Fourth Edition, W. B. Saunders Co., Philadelphia, PA, 1981, pp. 386-389.

Levy, Robert I., Lees, Robert S. and Fredrickson, Donald S., The nature of pre-beta (very low density) lipoproteins. J. Clin. Invest. 45: 63-77, 1966.

Lichenstein, Alice H. and Brecher, Peter, Properties of acyl CoA:cholesterol acyltransferase in rat liver. Topological localization and effects of detergents, albumin and polar steroids. J. Biol. Chem. 255: 9098-9104, 1980.

Lindgren, Frank T., The plasma lipoproteins: Historical developments and nomenclature. Ann. N. Y. Acad. Sci. 348: 1-15, 1980.

Lindgren, Frank T., Shen, Mason, Krauss, Ronald M. and Sargent, Thornton M. IV., Ultracentrifugation of HDL. In Report of the High Density Lipoprotein Methodology Workshop, Kenneth Lippel ed., NIH publication No. 79-1661, 1979, pp. 45-51.

Lipid Research Clinics Program, The Lipid Research Clinics coronary primary prevention trial results. I. Reduction in incidence of coronary heart disease. JAMA 251: 351-364, 1984a.

Lipid Research Clinics Program, The Lipid Research Clinics coronary primary prevention trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 251: 365-374, 1984b.

Lippiello, Patrick M. and Waite, Moseley, Kinetics and mechanism of phosphatidylcholine and cholesterol exchange between chylomicrons and high-density lipoproteins. Biochem. J. 215: 279-286, 1983.

Lombardo, D., Guy, O., Studies on the substrate specificity of a carboxyl esters hydrolase form human pancreatic juice II. Action on cholesterol esters and lipid soluble vitamin esters. Biochim. Biophys. Acta 611: 147-155, 1980.

Lund-Katz, Sissel and Phillips, Michael C., Location and motion of free cholesterol molecules in high density lipoprotein. Biochem. Biophys. Res. Comm. 100: 1735-1742, 1981.

Lund-Katz, Sissel and Phillips, Michael, Packing of cholesterol molecules in human high-density lipoproteins. Biochemistry 23: 1130-1138, 1984.

Macgee, Joseph, Ishikawa, Terry, Miller, William, Evans, Grace, Steiner, Paula and Glueck, Charles J., A micromethod for analysis of total plasma cholesterol using gas liquid chromatography. J. Lab. Clin. Med. 82: 656-662, 1973.

Macheboeuf, M., Recherches sur les phosphoaminolipides et les sterides du serum et du plasma sanguins. 1. Entrainement des phospholipides, des sterols et des sterides par les diverses fractions au cours du fractionnement des proteides du serum. Bull. Chim. Soc. Biol. 11: 268-293, 1929.

Macheboeuf, Michel and Vanaud, Henriette, Etudes su l'electrophorese des cenapses lipidoproteidiques du serum sanguin. Les cenapses acido-precipitables. Comp. Red. Soc. de Biol. 135: 1249-1251, 1941.

Maciejko, James J., Holmes, David R., Kottke, Bruce A., Zinsmeister, Alan R., Dinh, Dac M., Mao, Simon J. T., Apolipoprotein A-I as a marker of angiographically assessed coronary-artery disease. N. Eng. J. Med. 309: 385-389, 1983.

Maciejko, James J. and Mao, Simon J. T., Radioimmunoassay of apolipoprotein A-I. Application of a non-ionic detergent (Tween-20) and solid-phase staphylococcus. Clin. Chem. 28: 199-204, 1982.

Macy, I. C. and Kelly, H. J., The composition of milks. Bull. Natl. Res. Council (Washington) No. 254, 1953.

Mahley, Robert W., Alterations in plasma lipoproteins induced by cholesterol feeding in animals including man. In Disturbances in Lipid and Lipoprotein Metabolism. John M. Dietschy, Antonio M. Gotto, Jr. and Joseph A. Ontko eds., Williams and Wilkins, Baltimore, MD, 1978, pp. 181-197.

Mahley, Robert W. and Holcombe, Kathleen S., Alterations of the plasma lipoproteins and apoproteins following cholesterol feeding in the rat. J. Lipid Res. 18: 314-324, 1977a.

Mahley, R. W. and Innerarity, T. L., Interaction of canine and swine lipoproteins with the low density lipoprotein receptor of fibroblasts as correlated with heparin/manganese precipitability. J. Biol. Chem. 252: 3980-3986, 1977b.

Mahley, Robert W., Innerarity, Thomas L., Pitas, Robert E., Weisgraber, Karl H., Brown, Judith H. and Gross, Erhard, Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification of arginyl residues in arginine-rich and B apoproteins. J. Biol. Chem. 252: 7279-7287, 1977c.

Mahley, Robert W., Innerarity, Thomas L., Weisgraber, Karl H. and Fry, Donald L., Canine hyperlipoproteinemia and atherosclerosis. Am. J. Pathol. 87: 205-226, 1977d.

Mahley, Robert W., Weisgraber, Karl H. and Innerarity, Thomas, Canine lipoproteins and atherosclerosis II. Characterization of the plasma lipoproteins associated with atherogenic and nonatherogenic hyperlipidemia. Circ. Res. 35: 722-733, 1974.

Malinow, M. R., Hojman, D. and Pellegrino, A., Different methods for the experimental production of generalized atherosclerosis in the rat. Acta Cardiol. 9: 480-497, 1954.

Manninen, V. and Malkonen, M., Serum lipoproteins in hypophysectomised and hypoxic rats. In High Density Lipoproteins and Atherosclerosis A. M. Gotto Jr., N.E. Miller and M. F. Oliver eds., Elsevier/North Holland Biomedical Press, New York, NY, 1978, pp. 213-218.

Manual of laboratory operations. Lipid research clinics programs. DHEW publication (NIH) 75-628, 1974.

Marcel, Yves L., Vezina, Camilla, Emond, Diane, Verdery, Roy B. and Milne, Ross W., High density lipoprotein subfractions isolated by heparin-Sepharose affinity chromatography and their role in cholesteryl ester transfer to very low density lipoproteins. J. Lipid Res. 22: 1198-1205, 1981.

Marcel, Y. L., Vezina, C., Teng, B. and Sniderman, A., Transfer of cholesterol esters between human high density lipoproteins and triglyceride-rich lipoproteins controlled by a plasma protein factor. Atherosclerosis 35: 127-133, 1980.

Marks, Ronald G., Analyzing Research Data. The Basics of Biomedical Research Methodology. Wadsworth Inc., Belmont, CA, 1982.

Marsh, Julian B. Lipoproteins in a nonrecirculating perfusate of rat liver. J. Lipid Res. 15: 544-550, 1974.

Marsh, Julian B., Apoproteins of the lipoproteins in a nonrecirculating perfusate of rat liver. J. Lipid Res. 17: 85-90, 1976.

Miettinen, Tatu A., Proia, Alan and McNamara, Donald J., Origins of fecal neutral steroids in rats. J. Lipid Res. 22: 485-495, 1981.

Miller, G. J. and Miller, N. E. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. Lancet 1: 16-19, 1975.

Miller, N. E., Weinstein, D. B. and Steinberg, D., Binding, internalization and degradation of high density lipoprotein by cultured normal fibroblasts. J. Lipid Res. 18: 438-450, 1977.

Miller, N. E., Weinstein, D. B. and Steinberg, D., Uptake and degradation of high density lipoprotein: Comparison of fibroblasts from normal subjects and from homozygous familial hypercholesterolemic subjects. J. Lipid Res. 19: 644-653, 1978.

Mishkel, Maurice A. and Crowther, Sandra M., Hypothyroidism an important cause of reversible hyperlipidemia. Clin. Chim. Acta 74: 139-151, 1977.

Morton, Richard E. and Zilversmit, Donald B., A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. J. Biol Chem. 256: 11992-11995, 1981.

Morton, Richard E. and Zilversmit, Donald B., Purification and characterization of lipid transfer protein(s) from human lipoprotein-deficient plasma. J. Lipid Res. 23: 1058-1067, 1982.

Muniz, Nehemias, Measurement of plasma lipoproteins by electrophoresis on polyacrylamide gel. Clin. Chem. 23: 1826-1833, 1977.

Nagelkerke, J. Fred, Havekes, Louis, van Hinsbergh, Victor W. M. and van Berker, Theo J. C., In vivo catabolism of biologically modified LDL. Arteriosclerosis 4: 256-264, 1984.

Narayan, K. Ananth, Lowered serum concentration of high density lipoproteins in cholesterol-fed rats. Atherosclerosis 13: 205-215, 1971.

Narayan, K. Ananth, Mary, G. Elizabeth S. and Kummerow, Fred A., Rat serum lipoproteins after partial hepatectomy. Proc. Soc. Exp. Biol. Med. 129: 6-12, 1968.

Nervi, F. O., Dietschy, J. M., Inhibition of hepatic cholesterogenesis by different lipoproteins. Circulation (Suppl. III) 50: 46, 1974.

Nestel, P. J. and Monger, E. A., Turnover of plasma esterified cholesterol in normocholesterolemic and hypercholesterolemic subjects and its relation to body build. J. Clin. Invest. 46: 967-974, 1967.

Nestel, P. J., Reardon, M. and Billington, T., In vivo transfer of cholesteryl esters from high density lipoproteins to very low density lipoproteins in man. Biochim. Biophys. Acta 573: 403-407, 1979.

Nichols, A. V. and Smith, L., Effect of very low-density lipoproteins on lipid transfer in incubated serum. J. Lipid Res. 6: 206-210, 1965.

Nicoll, Ann and Lewis, Barry, Evaluation of the role of lipoprotein lipase and hepatic lipase in lipoprotein metabolism: In vivo and vitro studies in man. Eur. J. Clin. Invest. 10: 487-495, 1980a.

Nicoll, A., Miller, N. E. and Lewis, B., High-density lipoprotein metabolism. Adv. Lipid Res. 17: 53-106, 1980b.

Nikkari, T., Schriebman, P. H., Ahrens, E. H. Jr., In vivo studies of sterol and squalene secretion by human skin. J. Lipid Res. 15: 563-573, 1974.

Nikkila, Esko A., Taskinen, Marja-Riitta, Rehunen, Seppo and Harkonen, Matti, Lipoprotein lipase activity in adipose tissue and skeletal muscle of runners: Relation to serum lipoproteins. Metabolism 27: 1661-1671, 1978.

Noel, Simon-Pierre and Rubinstein, David, Secretion of apolipoproteins in very low density and high density lipoproteins by perfused liver. J. Lipid Res. 15: 301-308, 1974.

Noma, A., Yokosuka, T. and Kitamura, K., Plasma lipids and apolipoproteins as discriminators for presence and severity of angiographically defined coronary artery disease. Atherosclerosis 49: 1-7, 1983.

Nomenclature Committee of the International Union of Biochemistry, In Enzyme Nomenclature. Academic Press. New York, NY, 1979.

Novikoff, Phyllis M. and Yam, Ana, Sites of lipoprotein particles in normal rat hepatocytes. J. Cell Biol. 76: 1-11, 1978.

Oester, Y. T., Adrenal medullary hormones and arteriosclerosis. Ann. N. Y. Acad. Sci. 72: 885-896, 1959.

Ohno, Yoshimi, Okazaki, Mitsuyo and Hara, Ichiro, Fractionation of human serum lipoproteins by high performance liquid chromatography I. J. Biochem. 89: 1675-1680, 1981.

Orci, Lelio, Carpentier, Jean-Louis, Perrelet, A., Anderson, Richard G. W., Goldstein, Joseph L. and Brown, Michael S., Occurance of low density lipoprotein receptors within large pits on the surface of human fibroblasts as demonstrated by freeze-etching. Exp. Cell Res., 113: 1-13, 1978.

Oschry, Yitzchak and Eisenberg, Shlomo, Rat plasma lipoproteins: Re-evaluation of a lipoprotein system in an animal devoid of cholesterol ester transfer activity. J. Lipid Res. 23: 1099-1106, 1982.

Packard, Christopher J. and Shepherd, James, The low-density-lipoprotein receptor pathway in familial hypercholesterolemia: Its role in pathogenesis and its response to therapy. Biochem. Soc. Trans. 11: 634-636, 1983.

Pasquali-Ronchetti, I., Calandra, S., Baccarani-Contri, M. and Montaguti, M., The ultrastructure of rat plasma lipoproteins. J. Ultrastruct. Res. 53: 180-192, 1975.

Patsch, Josef R., Gotto, Antonio M. Jr., Olivecrona, Thomas and Eisenberg, Shlomo, Formation of high density lipoprotein$_2$-like particles during lipolysis of very low density lipoproteins *in vitro*. Proc. Natl. Acad. Sci. 75: 4519-4523, 1978.

Patsch, W., Sailer, S. and Braunsteiner, H., An enzymatic method for the determination of the initial rate of cholesterol esterification in human plasma. J. Lipid Res. 17: 182-185, 1976.

Pattnaik, Nikhil M., Montes, Agustin, Hughes, L. Barry and Zilversmit, Donald B., Cholesteryl ester exchange protein in human plasma isolation and characterization. Biochim. Biophys. Acta 530: 428-438, 1978.

Pattnaik, Nikhil M. and Zilversmit, Donald B., Interaction of cholesteryl ester exchange protein with human plasma lipoproteins and phospholipid vesicles. J. Biol. Chem. 254: 2782-2786, 1979.

Perret, Bertrand-Pierre, Eisenberg, Shlomo, Chajek-Shauel, Tova, Deckelbaum, Richard and Olivecrona, Thomas, Free cholesterol distribution during in vitro lipolysis of rat plasma very low density lipoprotein: Lack of a role for blood and heart cells. Europ. J. Clin. Invest. 13: 419-428, 1983.

Phillips, Gerald B., Castelli, William P., Abbott, Robert D. and McNamara, Patricia M., Association of hyperestrogenemia and coronary heart disease in men in the Framingham cohort. Am. J. Med. 74: 863-869, 1983.

Phillips, M. C., McLean, L. R., Stoudt, G. W. and Rothblat, G. H., Mechanism of cholesterol efflux from cells. Atherosclerosis 36: 409-422, 1980.

Pihl, A., The effect of dietary fat on the intestinal cholesterol absorption and on the cholesterol metabolism in the liver of rats. Acta Physiol. Scand. 34: 183-217, 1955.

Pinon, Jean-Claude, Bridoux, Anne-Marie and Leudat, Marie-Helene, Initial rate of cholesterol esterification associated with high density lipoproteins in human plasma. J. Lipid Res. 21: 406-414, 1980.

Quarfordt, S. H., Jain, R. S., Jakoi, L., Robinson, S. and Shelburne, F., The heterogeneity of rat high density lipoproteins. Biochem. Biophys. Res. Comm. 83: 786-793, 1978.

Quintao, E., Grundy, S. M., Ahrens, E. H., Effects of dietary cholesterol on the regulation of total body cholesterol in man. J. Lipid Res. 12: 233-247, 1971.

Redgrave, T. G., Formation of cholesterol ester-rich particulate lipid during metabolism of chylomicrons. J. Clin. Invest. 49: 465-471, 1970.

Rehnborg, Carl S. and Nichols, Alex V., The fate of cholesteryl esters in human serum incubated in vitro at 38°. Biochim. Biophys. Acta 84: 596-603, 1964.

Reichl, D., Postiglione, A., Myant, N. B., Pflug, J. J. and Press, M., Observations on the passage of apoproteins from plasma lipoproteins into peripheral lymph in two men. Clin. Sci. Mol. Med. 49: 419-426, 1975.

Reichl, D., Simons, L. A., Myant, N. B., Pflug, J. J. and Mills, G. L., The lipids and lipoproteins of human peripheral lymph, with observations on the transport of cholesterol from plasma and tissues into lymph. Clin. Sci. Mol. Med. 45: 313-329, 1973.

Reiser, Raymond and Sidelman, Zvi, Control of serum cholesterol homeostasis by cholesterol in the milk of the suckling rat. J. Nutr. 102: 1009-1016, 1972.

Remington, Richard D. and Schork, M. Anthony, Statistics with Applications to the Biological and Health Sciences, Prentice Hall Inc., Englewood Cliffs, NJ, 1970.

Report of the Working Group on Arteriosclerosis of the National Heart, Lung and Blood Institute, In Arteriosclerosis Volume 2. NIH Publication No. 82-2035, 1981, pp. 5-9.

Richmond, W., Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem. 19: 1350-1356, 1973.

Riemersma, R. A., Coronary artery disease: Raised cholesterol or triglycerides? Int. J. Cardiol. 5: 193-194, 1984.

Riesen, W. F., Mordasini, R., Salzmann, C., Thelar, A. and Gurtner, H. P., Apoproteins and lipids as discriminators of severity of coronary heart disease. Atherosclerosis 37: 157-162, 1980.

Rodwell, Victor W., Nordstrom, Jeffrey L. and Mitschelen, Johnathon J., Regulation of HMG CoA reductase. Adv. Lipid Res. 14: 1-74, 1976.

Roheim, Paul S., Gidez, Lewis I. and Eder, Howard A., Extrahepatic synthesis of lipoproteins of plasma and chyle: Role of the intestine. J. Clin. Invest. 45: 297-300, 1966.

Roheim, Paul S., Haft, David E., Gidez, Lewis I., White, Abraham and Eder, Howard A., Plasma lipoprotein metabolism in perfused rat livers. II. Transfer of free and esterified cholesterol into the plasma. J. Clin. Invest. 42: 1277-1285, 1963.

Rose, Herbert G. and Ellerbe, Polly, Lipoprotein products of lecithin:cholesterol acyltransferase and cholesteryl ester transfer. Biochim. Biophys. Acta 712: 547-556, 1982.

Ross, Russell, The arterial wall and atherosclerosis. Ann. Rev. Med. 30: 1-15, 1979.

Ross, Russell, The pathogenesis of atherosclerosis. In Coronary Artery Disease. William P. Santamore and Alfred A. Bove eds., Urban and Schwarzenberg, Baltimore, MD, 1982, pp. 139-147.

Rosseneu-Motreff, M. Y., Blaton, V., Declercq, B. and Peeters, H., The contribution of bound fatty acids to the heterogeneity of BSA. In Protides of the Biological Fluids Vol. 17. H. Peeters ed., Pergamon Press, New York, NY, 1969, pp. 397-400.

Royal College of General Practitioners, Further analysis of mortality in oral contraceptive use. Lancet 1: 541-546, 1981.

Rudel, Lawrence L., Lee, Jason A., Morris, Manford D. and Felts, James M., Characterization of plasma lipoproteins separated and purified by agarose-column chromatography. Biochem. J. 139: 89-95, 1974.

Sabesin, S. M., Ultrastructural aspects of the intracellular assembly transport and exocytosis of chylomicrons by rat intestinal absorptive cells, In Lipid Absorption Biochemical and Clinical Aspects. Karl Rommel, H. Goebell and R. Bohmer eds., University Park Press, Baltimore, MD, 1976, pp. 113-145.

Schaeffer, E. J., Anderson, D. W., Brewer, H. B. Jr., Levy, R. I., Danner, P. N. and Blackwelder, W. C., Plasma-triglycerides in regulation of H.D.L.-cholesterol levels. Lancet 2: 391-392, 1978a.

Schaefer, Ernst J., Eisenberg, Shlomo and Levy, Robert I., Lipoprotein apoprotein metabolism. J. Lipid Res. 19: 667-687, 1978b.

Schjeide, O. A., Rivin, A. U. and Yoshino, J., Uptake of lipid stains by lipids and serum lipoproteins. Am. J. Clin. Pathol. 39: 329-341, 1963.

Schonfeld, Gustav, Bell, Elliott and Alpers, David H., Intestinal apoproteins during fat absorption. J. Clin. Invest. 61: 1539-1550, 1978.

Schonfeld, Gustav, Lees, Robert S., George, P. K. and Pfleger, Barbara, Assay of total plasma apolipoprotein B concentration in human subjects. J. Clin. Invest. 53: 1458-1467, 1974a.

Schonfeld, Gustav and Pfleger, Barbara, The structure of human high density lipoprotein and the levels of apolipoprotein A-I in plasma as determined by radioimmunoassay. J. Clin. Invest. 54: 236-246, 1974b.

Schultz, S. G. and Strecker, C. K., Cholesterol and bile salt influxes across brush border of rabbit jejunum. Am. J. Physiol. 220: 59-65, 1971.

Schwartz, Charles C., Berman, Mones, Vlahcevic, Z. Reno and Swell, Leon, Multicompartmental analysis of cholesterol metabolism in man. J. Clin. Invest. 70: 863-876, 1982.

Schwartz, Charles C., Vlahcevic, Z. Reno, Halloran, L. Gregg and Swell, Leon, An in vivo evaluation in man of the transfer of esterified cholesterol between lipoproteins and into the liver and bile. Biochim. Biophys. Acta 663: 143-162, 1981.

Scow, Robert O., Hamosh, Margit, Blanchette-Mackie, E. S. Joan and Evans, Anthony J., Uptake of blood triglyceride by various tissues. Lipids 7: 497-505, 1972.

Shefer, S., Hauser, S., Lapar, V. and Mosbach, E. H., Regulatory effects of dietary sterols and bile acids on rat intestinal HMG Co A reductase. J. Lipid Res. 14: 400-405, 1973.

Shelburne, Frank, Hanks, John, Meyers, William and Quarfordt, Steven, Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. J. Clin. Invest. 65: 652-658, 1980.

Shen, B. W., Scanu, A. M. and Kezdy, F. J., Structure of human serum lipoproteins inferred from compositional analysis. Proc. Natl. Acad. Sci. 74: 837-841, 1977.

Shireman, Rachel B. and Williams, Debbie, Evaluation of the rapid micromethod for ultracentrifugal separation of labeled plasma lipoproteins. Lipids 18: 636-640, 1983.

Shore, Virgie G., Shore, Bernard and Hart, Roger G., Changes in apolipoproteins and properties of rabbit very low density lipoproteins on induction of cholesteremia. Biochemistry 13: 1579-1584, 1974.

Sigurdsson, G., Nicoll, A. and Lewis, B., Conversion of very low density lipoprotein to low density lipoprotein. A metabolic study of apolipoprotein B kinetics in human subjects. J. Clin. Invest. 56: 1481-1490, 1975.

Sigurdsson, G., Nicoll, A. and Lewis, B., The metabolism of low density lipoprotein in endogenous hypertriglyceridaemia. Europ. J. Clin. Invest. 6: 151-158. 1976.

Simmonds, Wilfred, J., Hofmann, Alan F. and Theodor, Emanuel, Absorption of cholesterol from a micellar solution: Intestinal perfusion studies in man. J. Clin. Invest. 46: 874-890, 1967.

Siperstein, Marvin D., Guest, Joanne M., Studies on the site of the feedback control of cholesterol synthesis. J. Clin. Invest. 39: 642-652, 1960.

Slone, Dennis, Shapiro, Samuel, Kaufman, David W., Rosenberg, Lynn, Miettinen, Olli S. and Stolley, Paul D., Risk of myocardial infarction in relation to current and discontinued use of oral contraceptives. N. Engl. J. Med. 305: 420-424, 1981.

Smith, Louis C., Pownall, Henry J. and Gotto, Antonio M. Jr., The plasma lipoproteins: Structure and metabolism. Ann. Rev. Biochem. 47: 751-777, 1978.

Sniderman, A., Teng, B., Vezina, C. and Marcel, Y. L., Cholesterol ester exchange between human plasma high and low density lipoproteins mediated by a plasma protein factor. Atherosclerosis 31: 327-333, 1978.

Sniderman, Allan D., Wolfson, Christina, Teng, Babie, Franklin, Frank A., Bachorik, Paul S. and Kwiterovich, Peter O., Association of hyperapobetalipoproteinemia with endogenous hypertriglyceridemia and atherosclerosis. Ann. Int. Med. 97: 833-839, 1982.

Spady, David K., Bilheimer, David W. and Dietschy, John M., Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. Proc. Natl. Acad. Sci. 80: 3499-3503, 1983.

Sperry, Warren M. and Webb, Merrill, A revision of the Schoenheimer-Sperry method for cholesterol determination. J. Biol. Chem. 187: 97-106, 1950.

Steele, Bernard W., Koehler, Donald F., Azar, Miquel, M., Blaszkowski, Thomas P., Kuba, Kanta and Dempsey, Mary E., Enzymatic determination of cholesterol in high-density-lipoprotein fractions prepared by a precipitation technique. Clin. Chem. 22: 98-101, 1976.

Stein, O., Rachmilewitz, D., Sanger, L., Eisenberg, S. and Stein, Y., Metabolism of iodinated very low density lipoprotein in the rat. Autoradiographic localization in the liver. Biochim. Biophys. Acta. 360: 205-216, 1974.

Stein, O. and Stein, Y., Bovine aortic endothelial cells display macrophage-like properties towards acetylated ^{125}I-labeled low density lipoprotein. Biochim. Biophys. Acta. 620: 631-635, 1980.

Stokke, K. T. and Norum, K. R., Determination of lecithin:cholesterol acyltransfer in human blood plasma. Scand. J. Clin. Invest. 27: 21-27, 1971.

Swaney, John B., Mechanisms of protein-lipid interaction. Association of apolipoproteins A-I an A-II with binary phospholipid mixtures. J. Biol. Chem. 255: 8791-8797, 1980.

Swaney, John B., Reese, Heidi and Eder, Howard A., Polypeptide composition of rat high density lipoprotein characterization by SDS-gel electrophoresis. Biochem. Biophys. Res. Comm. 59: 513-519, 1974.

Swell, L., Flick, D. F. and Field, H. Jr., Role of fat and fatty acid in absorption of dietary cholesterol. Am. J. Physiol. 180: 124-128, 1955.

Tabacik, C., Aliau, S. and Crastes de Paulet, A., Digitonin-precipitable sterols as a measure of cholesterol biosynthesis: Contradictory results. Lipids 18: 641-649, 1983.

Tall, Alan R., Abreu, Edith and Shuman, Janet, Separation of a plasma phospholipid transfer protein from cholesterol ester/phospholipid exchange protein. J. Biol. Chem. 258: 2174-2180, 1983a.

Tall, Alan R., Forester, Lynne R. and Bongiovanni, Gail L., Facilitation of phosphatidylcholine transfer into high density lipoproteins by an apolipoprotein in the density 1.210-1.26 g/ml fraction of plasma. J. Lipid Res. 24: 277-289, 1983b.

Tall, A. R. and Small, D. M., Current concepts plasma high-density lipoproteins. N. Eng. J. Med. 299: 1232-1236, 1978.

Tayeau, F. and Nivet, R., Esterification du cholesterol et lipoproteines seriques. In Biochemical Problems of Lipids. G. Popjak and E. LeBreton eds., Interscience Publishers Inc., New York, NY, 1956, pp. 365-370.

Thompson, G. Marie and Roy, A. V., The dextran sulfate-$MgSO_4$ precipitation as a method for quantitation of high-density lipoprotein cholesterol. Am. J. Clin. Pathol. 75: 267-269, 1981.

Thornton, John, Symes, Carol and Heaton, Kenneth, Moderate alcohol intake reduces bile cholesterol saturation and raises HDL cholesterol. Lancet 2: 819-821, 1983.

Tollin, C., Ericsson, M., Johnson, O., Backman, C., Jacobsson, K. A., Osterman, G., and Billstrom, A., Decreased removal of triglycerides from the blood a mechanism for the hypertriglyceridemia in male patients with coronary artery disease. Int. J. Cardiol. 5: 185-192, 1984.

Traber, Maret, Kallman, Brandon and Kayden, Herbert J., Localization of the binding sites of native and acetylated low density lipoprotein (LDL) in human monocyte-derived macrophages. Exp. Cell Res. 148: 281-292, 1983.

Turley, Stephen D., Andersen, John M., Dietschy, John M., Rates of sterol synthesis and uptake in the major organs of the rat. J. Lipid Res. 22: 551-569, 1981a.

Turley, Stephen D., Dietschy, John M., The contribution of newly synthesized cholesterol to biliary cholesterol in the rat. J. Biol. Chem. 256: 2348-2446, 1981b.

Vander Heiden, Gary L., Barboriak, Joseph J., Sasse, Edward A. and Yorde, Donal E., Correlation of the extent of coronary occlusion with apo B levels. Application of a new enzyme immunoassay technique for apo B. Atherosclerosis 50: 29-33, 1984.

Van Lenten, Brian J., Melchior, George W. and Roheim, Paul S., Lipoprotein metabolism in the ovariectomized rat. J. Lipid Res. 24: 1475-1484, 1983.

Vansteveninck, J., Weed, R. I. and Rothstein, A., Localization of erythrocyte membrane sulfhydryl groups essential for glucose transport. J. Gen. Physiol. 48: 617-632, 1965.

Vedeler, Christian A., Nyland, Harald and Matre, Roald, In situ characterization of the foam cells in early human atherosclerotic lesions. Acta Path. Microbiol. Immunol. Scand. 92: 133-137, 1984.

Vela, Benito A. and Acevedo, Hernan F., Urinary cholesterol. I. Isolation and characterization of "free" cholesterol in normal and pregnant subjects. J. Clin. Endocr. 29: 1251-1258, 1969.

Velick, Sidney F., Coenzyme binding and the thiol groups of glyceraldehyde-3-phosphate dehydrogenase. J. Biol. Chem. 203: 563-573, 1953.

Walker, A. R. P. and Walker, B. Faith, High high-density-lipoprotein cholesterol in African children and adults in a population free of coronary heart disease. Brit. Med. J. 2: 1336-1338, 1978.

Walton, K. W., Scott, P. J., Dykes, P. W. and Davies, J. W. L., The significance of alterations in serum lipids in thyroid dysfunction. II. Alterations of the metabolism of ^{131}I low-density lipoproteins in hypothyroidism and thyrotoxicosis. Clin. Sci. 29: 217-238, 1965.

Warnick, G. Russell and Albers, John J., HDL cholesterol quantitation: Comparison of six precipitation methods and ultracentrifugation. In Report of the High Density Lipoprotein Methodology Workshop. Kenneth Lippel ed., NIH Publication No. 79-1661, 1979a, pp. 53-69.

Warnick, G. Russell, Cheung, Marian C. and Albers, John J., Comparison of current methods for high-density lipoprotein cholesterol quantitation. Clin. Chem. 25: 596-604, 1979b.

Watson, W. C., Paper electrophoresis of serum lipoproteins in the normal and hypercholesterolaemic rat. Brit. J. Expt. Pathol. 42: 131-134, 1961.

Weiner, I. M. and Lack, L., Bile salt absorption: Enterohepatic circulation. In Handbook of Physiology. Vol. III. Alimentary Canal. C. F. Code ed., Am. Physiol. Soc., Washington D. C., 1968, pp. 1439-1455.

Weinstein, D. B., Schreiber, J. R. and Erickson, G. F., High density lipoprotein catabolism provides a cholesterol pool for hormone stimulated synthesis of progesterones by cultured rat ovarian cells. Circulation 62, Suppl. III: 212, 1980.

Weisgraber, Karl H., Mahley, Robert W. and Assman, Gerd, The rat arginine-rich apoprotein and its redistribution following injection of iodinated lipoproteins into normal and hypercholesterolemic rats. Atherosclerosis 28: 121-140, 1977.

Westergaard, H. and Dietschy, J. M., The mechanism whereby bile acid micelles increase the fatty acid and cholesterol uptake into the intestinal mucosal cell. J. Clin. Invest. 58: 97-108, 1976.

Wexler, Bernard C., Spontaneous coronary arteriosclerosis in repeatedly bred male and female rats. Circ. Res. 14: 32-43, 1964.

Whayne, Thomas F., Alaupovic, Petar, Curry, Michael D., Lee, Elisa T., Anderson, Paul S. and Schechter, Eliot, Plasma apolipoprotein B and VLDL-, LDL- and HDL-cholesterol as risk factors in the development of coronary artery disease in male patients examined by angiography. Atherosclerosis 39: 411-424, 1981.

Wheater, P. R., Burkitt, H. G. and Daniels, V. G., Functional Histology, Churchill Livingston, New York, NY, 1979, pp. 32-35.

Wheeler, Henry O., Secretion of bile acids by the liver and their role in the formation of hepatic bile. Arch. Intern. Med. 130: 533-541, 1972.

Windler, Eberhard, Chao, Yu-Sheng and Havel, Richard J., Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. J. Biol. Chem. 255: 5475-5480, 1980a.

Windler, Eberhard E. T., Kovanen, Petri T., Chao, Yu-Sheng, Brown, Michael S., Havel, Richard J. and Goldstein, Joseph L. The estradiol-stimulated lipoprotein receptor of rat liver. A Binding site that mediates uptake of rat lipoproteins containing apoproteins B and E. J. Biol. Chem. 255: 10464-10471, 1980b.

Windmueller, H. G. and Levy, Robert I., Production of β-lipoprotein by intestine in the rat. J. Biol. Chem. 243: 4878-4884, 1968.

Wissler, R. W., Eilert, M. L., Schroeder, M. A. and Cohen, L., Production of lipomatous and atheromatous arterial lesions in the albino rat. Arch. Pathol. 57: 333-351, 1954.

Wu, Ai-Lien and Windmueller, Herbert G., Relative contributions by liver and intestine to individual plasma apolipoproteins in the rat. J. Biol. Chem. 254: 7316-7322, 1979.

Young, Patricia M. and Brecher, Peter, Cholesteryl ester transfer from phospholipid vesicles to human high density lipoproteins. J. Lipid Res. 22: 944-954, 1981.

Zilversmit, Donald B., A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride-rich lipoproteins. Circ. Res. 33: 633-638, 1973.

Zilversmit, D. B., Hughes, L. B. and Balmer, Joanne, Stimulation of cholesterol ester exchange by lipoprotein-free rabbit plasma. Biochim. Biophys. Acta 409: 393-398, 1975.

APPROVAL SHEET

The dissertation submitted by Isaac Bruce Rosenzweig has been read and approved by the following committee:

Joseph R. Davis, M.D., Ph.D., Director
Professor of Pharmacology
Loyola University Medical Center

Edward Bermes, Jr., Ph.D.
Professor of Biochemistry and Biophysics
and Acting Chairman of Pathology
Loyola University Medical Center

Silas N. Glisson, Ph.D.
Associate Professor of Pharmacology and Anesthesiology
Loyola University Medical Center

Mary Druse Manteuffel, Ph.D.
Associate Professor of Biochemistry and Biophysics
Loyola University Medical Center

Louis D. Van de Kar, Ph.D.
Assistant Professor of Pharmacology
Loyola University Medical Center

The final copies have been examined by the director of the dissertation and the signature which appears below verified the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

Mar. 29, 1985
Date

Joseph R. Davis, M.D., Ph.D.
Director's Signature