Title

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Publication Date

1984-04-01

Thesis/dissertation

LBL-17826

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

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(Ph.D. Thesis)

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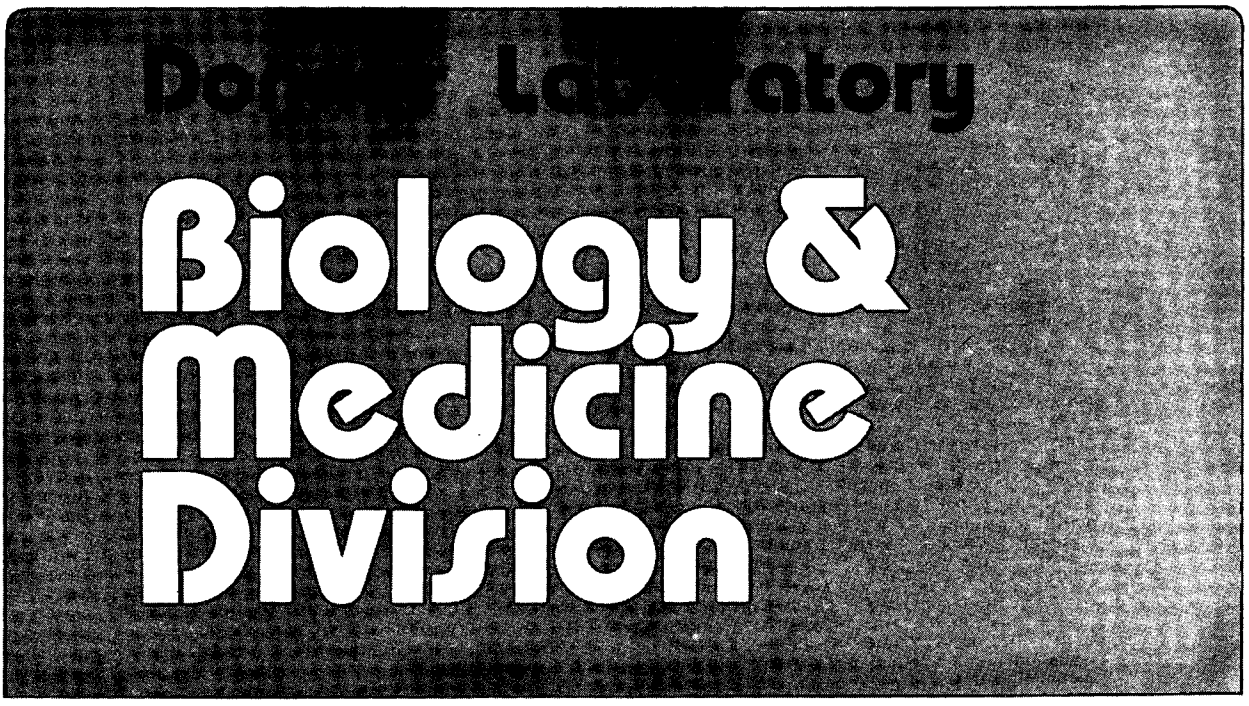

Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

LBL-17826

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CHARACTERIZATION OF BIOPHYSICAL PROPERTIES OF BABOON LIPOPROTEINS: MODULATION OF DIETARY FAT AND CHOLESTEROL

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April 1984

Characterization of Biophysical Properties of Baboon Lipoproteins: Modulation by Dietary Fat and Cholesterol

John Babiak

Abstract

The serum lipoproteins of pedigreed and non-pedigreed baboons (Papio cynocephalus) fed diets containing differing types and amounts of fat and varying amounts of cholesterol were examined by analytic ultracentrifugation, gradient gel electrophoresis, density gradient ultracentrifugation, sodium dodecyl sulfate-polyacrilamide electrophoresis, electron microscopy, and standard protein and lipid composition assays. The objectives of these studies were to characterize the lipoproteins of the baboon, to observe how concentrations and physical-chemical properties of the lipoproteins are modulated by dietary fat and cholesterol and by genetic background and to describe the suitability of the baboon as an animal model of human lipoprotein metabolism.

The results of the studies indicate that baboon high density lipoproteins (HDL), though higher in total serum concentration than human HDL, are remarkably similar to human HDL. The HDL of both species contain apolipoproteinA-I as its major protein and demonstrate considerable polydispersity. Thus, the HDL of both species consist of five discrete subpopulations which are separable by density gradient ultracentrifugation. The five subpopulations of baboon HDL are each similar to their human

counterparts in terms of hydrated density, particle size, chemical composition and apolipoprotein content.

The concentration of baboon HDL is increased by saturated fat (relative either to polyunsaturated fat or to a low fat diet), but is decreased by the addition of cholesterol to a diet high in saturated fat. Similar results have been obtained for human HDL concentrations.

Serum concentrations of low density lipoproteins (LDL) tend to be lower in baboons, but the physical-chemical properties of the LDL of both baboons and humans are quite comparable. The LDL of both species contains apolipoproteinB as their major apolipoprotein and exhibit considerable polydispersity in particle size. LDL of both species consists of seven discrete subpopulations.

Linear correlation coefficients between concentrations of lipoprotein classes and/or subpopulations were calculated since such correlations may reflect metabolic relationships. The correlations calculated for baboon lipoprotein concentrations are comparable to similar correlations calculated for lipoprotein concentrations made in humans.

Epidemiological studies have indicated relationships between concentrations of lipoprotein components and the risk of coronary heart disease and atherosclerosis in humans. The analytical and statistical data presented in this dissertation indicate that the baboon is a good model for human lipoprotein structure and metabolism and for study

of the role of lipoproteins in the development of atherosclerosis.

Dedication

This dissertation is dedicated to my research adviser, Professor Alex Nichols. During the four years I have worked in his laboratory, he has been a friend, a colleague and a teacher. I will always remember and appreciate his help and guidance.

Acknowledgements

I would like to take this opportunity to acknowledge and thank the following people who have influenced my life during the seven years I have spent in California:

Elaine Gong and Pat Blanche, who, together, taught me everything I know about working with lipoproteins. They never ceased to help me with my problems, tactfully correct my mistakes and keep me smiling. In addition, Elaine is personally responsible for a lot of the data in this dissertation.

Trudy Forte, Ron Krauss, Frank Lindgren and Virgie Shore, who were always willing to answer questions and remained supportive, even though I was working with baboons. Trudy Forte and Bob Nordhousen provided the electron micrographs used in this dissertation. Virgie Shore provided some of the SDS-polyacrilamide gel electrophoresis data presented in this dissertation.

Laura Glines, Gerry Adamson and Joseph Orr, who performed the analytic ultracentrifugation runs described in this dissertation. Laura made an enormous effort to schedule analytic runs for me and also digitized a large number of these runs. Gerry patiently dealt with all my questions which were either extremely trivial or strangely obscure.

Linda Stimson and Linda Abe, who provided a safe, non-scientific haven in the middle of Donner Lab. They both

have spent countless hours typing and retyping papers for me (but not this dissertation) and let me use their typewriters (before I discovered UNIX).

Virginia Obie and Charlie Mae Fuller, who helped me locate unique lab glassware and centrifuge prep caps.

Cris Giotas, Dennis Duncan, Zahra Shahrokh and Jim Hunter, who made sure that 261 Donner was a unique place to work.

Henry McGill, Glen Mott and Evelyn Jackson, at the Southwest Foundation for Research and Education, who sent me all those baboon serum samples that will get me a Ph.D. Henry McGill has been extremely friendly and supportive. I have tremendously enjoyed collaborating with him and look forward to working with him again.

Richard Dalven and Howard Shugart, who gave me the opportunity to teach in the Physics Department. Teaching, under Dr. Dalven's guidance, was one of the most rewarding experiences during the last seven years and helped me avoid starvation.

All the friends I have made at Berkeley, especially Rachelle Bergmann and Paul Bash, who did all those things friends are supposed to do.

All the members of the St. Michael Orthodox Church in Danville, CA, who have given me friendship, support and love, and substituted for the family I left in NJ.

My mother, who did not want me to go to California, but let me go, anyway.

Last of all, I wish to acknowledge my best friend, Susan Pelkey, who did everything possible to help me get this dissertation finished. She typed, proofread, collated, numbered pages, pointed out missing tables, and, even, read this thing. She, with her love, has given me happiness, confidence and the desire to complete this project. I know that, without her, I would not have finished this dissertation on time.

My research was supported by NIH NHLBI National Research Service Award Grant HL07279.

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Chapter I: Introduction

Epidemiological studies have established a positive correlation between the risk of coronary heart disease and serum low density lipoprotein (LDL) concentrations, while high density lipoprotein (HDL) concentrations were found to exhibit a negative correlation (1-7). These results have led to numerous studies in animals examining the development of atherosclerosis in major arteries concommitant with modification of lipoprotein metabolism induced by diet (8-14), drugs (15), hypothyroidism (16,17), diabetes (18) and genetic background (19-21).

The baboon (Papio cynocephalus) is one of several non-human primates which has been studied as a model for experimentally-induced atherosclerosis (22-28). In response to high levels of dietary saturated fat and cholesterol (for a period of 2 years) the baboon develops fatty streaks and, occasionally, fibrous plaques; the extent and severity of the disease, however, are not as great as in other animal models (9). Studies investigating lipoprotein metabolism in the baboon have ranged from evaluations of the absorption of dietary cholesterol (29,30), to determinations of rates of endogenous synthesis (31) and turnover (32-34) of cholesterol.

The apolipoprotein moiety of baboon lipoproteins has been studied by several investigators (8,35-41). In this regard, the baboon exhibits an array of apolipoproteins which compares quite well with their human counterparts in

terms of molecular weight (8) and immunoreactivity (18). In addition, baboon apoA-I and apoA-II exhibit considerable homology with the corresponding human apolipoproteins (8,35,37,42).

The physical-chemical properties of baboon lipoproteins, however, have not been well characterized. The chemical composition of total lipoprotein classes (VLDL, LDL and HDL) has been examined by two investigators (18,35). One investigator (43) has examined the properties of subfractionated HDL (HDL_2 and HDL_3), but used density gradient ultracentrifugal techniques designed for human HDL. While two subfractions were obtained according to their hydrated density, the biophysical and/or metabolic significance of the two subfractions were not examined.

The identification and study of discrete subpopulations within human lipoprotein classes has been the subject of numerous investigations. Anderson et al (44) demonstrated the existence of three major subpopulations of human HDL which were separable by density gradient ultracentrifugation and showed that concentrations of these individual subpopulations correlate independently with the concentrations of other serum lipoprotein components. In addition, they noted that the serum concentrations of some of these subpopulations are related to the individual's age and sex. Later, Blanche et al (45), using the method of gradient gel electrophoresis, demonstrated the presence of a total of five subpopulations of human HDL. Studies of the modulation of

the concentrations of these human HDL subpopulations by diet, exercise and other factors (46,47) are preliminary.

Evidence for the existence of, possibly, seven subpopulations within human LDL (defined on the basis of particle size) has been recently presented by Krauss and Burke (48), utilizing gradient gel electrophoresis, density gradient ultracentrifugation and analytic ultracentrifugation. Previous studies of Krauss et al (49) demonstrated that concentrations of LDL of flotation rate $>S^{o}_{f}7$ correlate (with concentrations of other lipoprotein fractions) independently from the concentrations of LDL with flotation rate $<S^{o}_{f}7$. Other studies have indicated that LDL of different sizes may be removed from the plasma compartment at different rates (50). These results indicate metabolic differences may exist between at least some of the LDL subpopulations.

Studies of lipoproteins and lipoprotein metabolism in animals usually measure the properties (e.g. mean composition, mean size) of the major lipoprotein classes (VLDL, LDL and HDL), and sometimes subfractionate HDL into HDL_2 and HDL_3 (51-69). These studies have rarely examined or identified individual lipoprotein subpopulations. One major purpose of the experiments described in this dissertation was to characterize the major lipoprotein classes in the baboon and then to identify and characterize discrete subpopulations within these lipoprotein classes. The primary methods utilized were gradient gel electrophoresis (70), analytic ultracentrifugation (71), density gradient

ultracentrifugation (72), sodium dodecyl sulfate-polyacrilamide gel electrophoresis (73) and standard methods for determination of chemical composition of lipoproteins (74-77). A second purpose was to study how the concentrations of baboon lipoprotein subpopulations are modulated by the type and amount of dietary fat and the amount of dietary cholesterol. To these ends several experiments were performed.

Experiment I involved 46 (mainly pre-pubertal) baboons which had consumed a diet, containing 40% of total calories in the form of lard and 1.7 mg/Kcal of cholesterol, since weaning. This diet has previously been shown to induce atherosclerotic lesions in baboons within a period of 2 years (25). The progeny were the product of assortative matings of five sires on the basis of total serum cholesterol (matings of high total serum cholesterol males with high total serum cholesterol females and low total serum cholesterol males with low total serum cholesterol females) observed on the same diet. Analysis of the serum lipoproteins from these progeny provided the initial data identifying and characterizing five HDL subpopulations (on the basis of particle size, apolipoprotein and chemical compositions, hydrated density and flotation properties) in both hypercholesterolemic and hypocholesterolemic baboons (78). Preliminary information on LDL subfractions in these animals was also obtained (Chapter III).

Concentrations of the major lipoprotein classes (VLDL, IDL, LDL and HDL) and HDL subpopulations were determined for all the progeny in Experiment I. The concentrations of the lipoprotein components of each family were compared, as were statistical correlations among the concentrations of these lipoprotein species (Chapter IV).

At the conclusion of Experiment I, five progeny of one hypercholesterolemic sire were placed on a sequence of diets containing different amounts of fat and cholesterol. Examination of the serum of these progeny yielded information regarding the modulation of lipoprotein concentrations and particle sizes by dietary fat and/or cholesterol (Chapter V).

In Experiment II (Chapter VI), 24 randomly-selected baboons were fed a sequence of diets containing high levels of cholesterol (1.7 mg/Kcal), and high levels (40% of total calories) of either a predominantly saturated fat (coconut oil) or a polyunsaturated fat (corn oil). This experiment was, in fact, a follow-up of a previous study (60) involving the same baboons and the same diets (as well as two diets with the same amounts and types of fat, but no added cholesterol) which examined the differential effects of dietary fat and cholesterol on the concentrations of heparin-manganese chloride precipitable and non-precipitable cholesterol. The experiment reported in this dissertation, therefore, was an attempt to explain, in more detail, which serum lipoprotein components are affected by the degree of

saturation of dietary fat. In addition, the data from this experiment provided evidence (on the basis of particle size) for the presence of seven subpopulations within baboon LDL and confirmed the results of Experiment I, demonstrating the presence of five subpopulations within baboon HDL.

The data reported in Chapter VII are preliminary results of a study of lipoproteins within the serum of baboons after consuming one of four diets (differing in the amount of cholesterol and the degree of saturation of fat) for six years. These animals were studied extensively over the six year period (29,30,52,53) by other investigators and will eventually be examined for the development of atherosclerotic lesions, lecithin:cholesterol acyltransferase and lipoprotein lipase activities and serum lipids.

Chapter II: Methods and Materials

1. Experiments

1.1. Subjects - Experiment I

Experiment I was based on 46 colony-reared baboon progeny (Papio cynocephalus) of five sires that were selectively chosen and bred. Sires and dams were selected from a group of 264 male and 419 female baboons that were previously challenged for four months with an atherogenic diet enriched in cholesterol and saturated fat (in the form of lard). From this group of baboons were selected sires and dams with total serum cholesterol concentrations above or below the cumulative mean for each sex. These dams and sires were mated assortatively, based on their total serum cholesterol levels (high with high and low with low) in response to the atherogenic diet. Throughout all studies all baboons were housed and fed at the Southwest Foundation for Research and Education in San Antonio, Texas.

Table 1 lists the total serum cholesterol levels of the five sires on the atherogenic diet whose progeny were used in Experiment I. Also included are the number, sex and ages (at the time of examination) of the progeny produced by assortative matings of these sires. Two sires (1672 and 839) had total serum cholesterol concentrations (461 and 282 mg/dl, respectively) which were more than two standard deviations above the group mean for the male baboons tested.

Table 1. Sires and progeny used as subjects in Experiment I

Sire	Sire's Serum Cholesterol on LARD+CS diet (mg/dl)	Number of Progeny in Experiment I	Age and Sex of Progeny			
			<18 mo.	19-29 mo.	30-42 mo.	>42 mo.
1672	461	11 (8F, 3M)	8F,3M			
839	282	12 (4F, 8M)		4F, 2M	0F, 6M	
102	183	13 (10F, 3M)		4F, 2M	3F, 0M	3F, 1M
808	124	5 (3F, 2M)	3F; 2M			
959	110	5 (3F, 2M)		3F, 2M		

M: Male

F: Female

These two sires were mated with dams whose total serum cholesterol levels were at least one standard deviation above the mean for females. Two other sires (808 and 959) had total serum cholesterol levels (124 and 110 mg/dl, respectively) which were more than two standard deviations below the mean for males in the group examined. These two sires were mated with dams whose total serum cholesterol levels were at least one standard deviation below the mean for females. The last sire (102) had a total serum cholesterol level (183 mg/dl) which was only one standard deviation above the mean for males and was mated with dams whose total serum cholesterol concentrations were above the mean for females.

In all, eighteen of the progeny examined were male and twenty-eight were female. All progeny were between 12 and 56 months of age when examined. Since puberty in the baboon begins at approximately 42 months of age, most of the progeny were pre-pubertal. Consequently, we observed no sex or age-related effects in either lipoprotein concentrations or particle size distributions in this population of baboons.

1.2. Subjects - Experiment II

Twenty-four baboons (12 males and 12 females) were randomly selected. These were subjects in a previous diet study (60) that was similar in nature to the diet study performed in this dissertation (Experiment II). The baboons ranged in age from 3 to 5 years when the present experiment

was conducted. Thirteen baboons were feral; eleven were raised in captivity and were the progeny of different sires and dams. Six animals of each sex were randomly assigned to each of two blocks with similar age distributions within each block.

1.3. Diets and Experimental Design - Experiment I

All pedigreed progeny were weaned to the LARD+CS (lard with cholesterol) diet (Table 2, column 1) at 15 weeks of age and were maintained on this diet until examined. This diet was comprised of a mix of Purina Monkey Meal, NaCl, vitamins A and C, cholesterol (a combination of dried egg yolk and crystalline cholesterol) and enough lard to provide 40% of total calories from fat. Analysis of the fat in the LARD+CS diet by gas-liquid chromatography showed that it consisted of 40.8% saturated, 41.2% monounsaturated and 17.9% polyunsaturated fatty acids. Although this diet was high in saturated fat, there was sufficient linoleic acid (16% of total fat) to avoid an essential fatty acid deficiency in these animals. All baboons were offered 500g of the diet per day, an amount that from earlier studies (30) was sufficient to ensure adequate growth. All baboons were provided with water ad libitum and were kept in individual cages.

At the conclusion of the experiment, five progeny of sire 102 were fed a series of diets with differing amounts of lard and cholesterol, in the order outlined in Table 3.

Table 2. Nutrient composition and ingredients of diets.

Nutrients	LARD+CS	LARD-CS	CHOW	CORN+CS	COCO+CS
Carbohydrate (% Kcal)	39	40	62	39	39
Protein (%Kcal)	21	20	28	21	21
Fat (%Kcal)	40	40	10	40	40
Energy (Kcal/100 g)	377	377	329	377	377
Cholesterol (mg/Kcal)	1.7	0.03	0.03	1.7	1.7
Ingredients (% dry weight)					
Baboon chow*	0	0	100	0	0
Monkey Meal**	79.1	81.8		79.1	79.1
Egg yolk, dried	4.8	0		4.8	4.8
Cholesterol	0.6	0		0.6	0.6
Lard	14.3	17.0			
Corn oil				14.3	
Coconut oil					14.3
NaCl	1.1	1.1		1.1	1.1
Retinyl acetate	0.001	0.001		0.001	0.001
Ascorbic acid	0.01	0.01		0.01	0.01

* Purina Baboon Chow (Ralston Purina, St. Louis, MO.)

** Purina Monkey Meal 25-5045-6, a special mix with no added fat, dehydrated alfalfa, NaCl, ascorbic acid or retinyl acetate.

Table 3. Diet sequence followed by five selected progeny at conclusion of Experiment I

Period	Length of Time	Diet
1	weaning through age 12-56 mos.	LARD+CS*
2	6 weeks	LARD-CS**
3	12 weeks	CHOW***
4	6 weeks	LARD-CS
5	6 weeks	LARD+CS

* LARD+CS diet, column 1 of Table 2

** LARD-CS diet, column 2 of Table 2

*** CHOW diet, column 3 of Table 2

The data from only four of these five were analyzed and presented in this dissertation because the fifth baboon exhibited dietary responses which were markedly different from the other four.

1.4. Diets and Experimental Design - Experiment II

Two diets were prepared by mixing coconut oil or corn oil with a basic chow (Table 2, columns 4 and 5; the COCO+CS (coconut oil with cholesterol) and CORN+CS (corn oil with cholesterol) diets). Both diets contained identical levels of cholesterol (1.7 mg/Kcal) provided by dried egg yolk and crystalline cholesterol. Analyses of the oils by gas-liquid chromatography showed that the corn oil (Mazola, Best Foods Co., Atlanta, GA) contained 12.6% saturated, 24.9% monounsaturated and 62.4% polyunsaturated fatty acids. Coconut oil (white coconut oil, Lou Ana Foods, Inc., Opelousas, LA), contained 90.9% saturated, 6.7% monounsaturated and 1.4% polyunsaturated fatty acids. The mixed diets were pelleted and stored in a freezer. Each animal received 500g of food daily, water ad libitum and all were kept in individual cages.

The diet sequence for all baboons (randomly divided into two groups of twelve animals each) is shown in Table 4. All animals were maintained on a CHOW diet (Table 2, column 3) for at least 3 months prior to the start of the experiment. Each group was alternately fed the 2 diets (COCO+CS or CORN+CS) for periods of six weeks each. In the final six

Table 4. Diet sequence for Experiment II.

Period	Group I	Group II	Length of Time
0	CHOW*	CHOW	>3 mos.
1	COCO+CS**	CORN+CS***	6 weeks
2	CORN+CS	COCO+CS	6 weeks
3	COCO+CS	CORN+CS	6 weeks

* CHOW diet, column 3 of Table 2

** COCO+CS diet, column 5 of Table 2

*** CORN+CS diet, column 4 of Table 2

week period, each group consumed the same diet as eaten during the first period.

1.5. Subjects and Experimental Design - Experiment III

The baboons used in Experiment III were subjects in a long-term (6 year) study of the effects of the saturation of dietary fat and the amount of dietary cholesterol. This investigation was conducted by Dr. Glen Mott and associates at the Southwest Foundation for Research and Education in San Antonio, Texas. Their studies included examination of the extent of atherosclerotic involvement in arteries, the fat content of major organs, measures of serum lipids and serum lecithin:cholesterol acyltransferase activity. Preliminary results of these studies have been reported (29,30,52,53). Plasma from 36 of the 96 baboons in the study were kindly provided for studies included in this dissertation.

Ninety-six baboons (48 male and 48 female), produced by the matings of six sires were divided into four groups, each consisting of 24 animals. The members of each group were fed one of four diets for six years; each diet contained (by percent of calories) 40.7% fat, 19.8% protein and 39.5% carbohydrate, but differed in fatty acid composition and cholesterol content. Two diets included predominantly saturated fatty acids (P/S ratio = 0.26); one was high in cholesterol (0.9 mg/Kcal) and one was low in cholesterol (0.013 mg/Kcal). The other two diets included predominantly

polyunsaturated fatty acids (P/S ratio = 2.1); one was high in cholesterol (0.9 mg/Kcal) and one was low in cholesterol (0.013 mg/Kcal). Plasma from 36 of the baboons was examined.

2. Analytical Methods

2.1. Blood Collection and Preparation

Venous blood was collected into evacuated tubes from each baboon after an overnight fast and under ketamine anesthesia (10 mg/kg body weight of Vetalar; Parke, Davis and Company, Detroit, MI). Serum was separated by centrifugation in a refrigerated centrifuge. Sera were shipped on wet ice to the Donner Laboratory, University of California, Berkeley for analysis and arrived within 30 hours of collection. Penicillin and streptomycin (1 ml/dl; Gibco Laboratories, Grand Island, NY) were added to samples upon arrival to reduce the possibility of bacterial contamination during subsequent studies.

In some cases, plasma was utilized. This was obtained by collecting venous blood into evacuated tubes containing ethylenediamine-tetra-acetic acid (EDTA, 1mg/ml final concentration). All other conditions for sample preparation and handling were the same as described for serum.

The possible effects of lecithin:cholesterol acyltransferase activity during shipment and handling were investigated by shipping identical serum samples with and without the lecithin:cholesterol acyltransferase inhibitor 5,5'-dithiobis-(2-nitrobenzoic acid) added to a final concentration of 1.4mM. No difference was observed in either serum lipoprotein concentrations (measured by analytic ultracentrifugation) or lipoprotein particle size

distributions (examined by gradient gel electrophoresis) for paired samples from each of two female progeny processed with or without the lecithin:cholesterol acyltransferase inhibitor. Consequently, it appeared that (for overnight shipment on ice), there were no significant lecithin:cholesterol acyltransferase-induced changes in lipoprotein patterns in the samples studied.

2.2. Analytic Ultracentrifugation

Samples of serum from each baboon were adjusted to either d 1.065 or d 1.217 g/ml with NaCl or NaCl-NaBr solution, respectively, and subjected to preparative ultracentrifugation (Beckman 40.3 rotor, 114,000 x g, 24 hr, 15°C). The top 1 ml from each tube was pipetted to remove either the serum $d \leq 1.063$ or $d \leq 1.20$ g/ml lipoprotein fraction. These fractions were then analyzed by both analytic ultracentrifugation and gradient gel electrophoresis.

Ultracentrifugally isolated serum $d \leq 1.063$ and $d \leq 1.20$ g/ml lipoprotein fractions were studied by analytic ultracentrifugation by the method of Lindgren et al (71). Baboon lipoprotein concentrations within specific flotation intervals were calculated from computer-derived schlieren patterns using specific refractive increments empirically determined for human lipoprotein classes. This procedure was reasonable because of the similarity between the sizes and chemical compositions of human and baboon HDL and LDL (data in Tables 6 and 11). To estimate the specific

refractive increment for $F^{o}_{1.20}9\text{-}28$ lipoproteins a linear interpolation between the empirical specific refractive increments of human HDL and LDL was performed and indicated a correction of less than 2% in $F^{o}_{1.20}9\text{-}28$ concentrations when using the increment for human HDL. Such correction was well within the resolution obtained by analytic ultracentrifugation in determining serum concentrations of lipoproteins. All measurements derived from schlieren patterns were adjusted to standard conditions and fully corrected for concentration dependence.

For lipoproteins floating at d 1.20 g/ml, $F^{o}_{1.20}9\text{-}28$ lipoproteins, by definition, were measured as material within the $F^{o}_{1.20}9\text{-}28$ flotation interval, while whole HDL was measured as material within the $F^{o}_{1.20}0\text{-}9$ interval. HDL was further divided into subpopulations as previously defined (78, and Table 6 in this dissertation) by gradient gel electrophoresis, density gradient ultracentrifugation and analytic ultracentrifugation.

Concentrations of baboon HDL subpopulations were obtained by a three-component analysis similar to the method of Anderson et al (44). HDL-I (particles of size 100-125A and density 1.063-1.120 g/ml) concentrations were calculated assuming a peak flotation rate of $F^{o}_{1.20}5.17$ (see Table 6). HDL-II (particles of size 88-100A and density 1.120-1.135 g/ml) concentrations were calculated assuming a peak flotation rate of $F^{o}_{1.20}2.77$. Baboon HDL-III, HDL-IV and HDL-V (particles of size 72-88A and density 1.135-1.20 g/ml) were

treated as a single component ("HDL-III through HDL-V") with a peak flotation rate of $F^{o}_{1.20}1.56$.

For lipoproteins floating at d 1.063 g/ml, VLDL was defined as material within the $S^{o}_{f}20-400$ flotation interval, IDL within the $S^{o}_{f}12-20$ flotation interval and LDL within the $S^{o}_{f}5-12$ flotation interval. The portion of $F^{o}_{1.20}9-28$ lipoprotein material which could be isolated within the $d \leq 1.063$ g/ml serum fraction generally was measured within the $S^{o}_{f}0-5$ flotation interval.

2.3. Density Gradient Ultracentrifugation

Preliminary isolation of lipoprotein classes was performed by sequential ultracentrifugation at d 1.006 g/ml (or d 1.019 g/ml), d 1.063 g/ml and d 1.20 g/ml. Lipoprotein fractions derived in this manner often contained more than one lipoprotein class or multiple subpopulations. Therefore, several equilibrium density gradient ultracentrifugation runs were devised to obtain more discrete lipoprotein subfractions.

VLDL and IDL, isolated within the serum $d \leq 1.019$ g/ml fraction, were further separated by density gradient ultracentrifugation. A 2.5 ml aliquot of deionized water (d 0.9982 g/ml) was layered over 5.5 ml of the isolated fraction (previously dialyzed to d 1.006 g/ml) which, in turn, was layered over 4.0 ml of d 1.019 g/ml NaCl solution. This layered preparation was ultracentrifuged for 48 hours at 174,000 x g at 15°C in a Beckman SW 41 rotor. Twelve 1 ml

fractions were pipetted from each tube. The final background salt gradient ranged from 1.002 g/ml to 1.022 g/ml, as determined by refractometry.

To separate $F^{o}_{1.20}9\text{-}28$ lipoproteins from LDL, equilibrium density gradient ultracentrifugation was performed on isolated serum d 1.006-1.063 g/ml fractions by a method adapted from Shen et al (72). A 2.5 ml aliquot of d 1.028 g/ml NaCl-NaBr solution was layered over 2.0 ml of the isolated lipoprotein fraction (previously dialyzed to d 1.040 g/ml) which, in turn, was layered over a 2.5 ml aliquot of d 1.054 g/ml NaCl-NaBr solution. This layered preparation was ultracentrifuged for 42 hours at 174,000 x g at 15°C in a Beckman SW 45 rotor. Seven 1 ml fractions or fourteen 0.5 ml fractions were pipetted from each tube. The final background salt gradient ranged from 1.025 g/ml to 1.066 g/ml, as determined by refractometry.

A second density gradient ultracentrifugation method was applied to separate the LDL and $F^{o}_{1.20}9\text{-}28$ lipoproteins contained within the d 1.019-1.063 g/ml fraction. A 4 ml aliquot of d 1.028 g/ml NaCl-NaBr solution was layered over 4 ml of the isolated lipoprotein fraction (previously dialyzed to d 1.040 g/ml) which, in turn, was layered over a 4 ml aliquot of d 1.054 g/ml NaCl-NaBr solution. This layered preparation was ultracentrifuged for 48 hours at 174,000 x g at 15°C in a Beckman SW 41 rotor. Twelve 1 ml fractions were pipetted from each tube. The final background salt gradient ranged from 1.026 g/ml to 1.072 g/ml,

as determined by refractometry.

To subfractionate the HDL, equilibrium density gradient ultracentrifugation was performed on isolated and washed serum d 1.063-1.20 g/ml lipoprotein fractions. A 2.5 ml aliquot of d 1.085 g/ml NaCl-NaBr solution was layered over 2 ml of the isolated lipoprotein fraction (previously dialyzed to d 1.109 g/ml), which, in turn, was layered over a 2.5 ml aliquot of d 1.133g/ml NaCl-NaBr solution. This layered preparation was ultracentrifuged for 42 hours at 174,000 x g at 15°C in a Beckman SW 45 rotor. Fourteen 0.5 ml fractions were pipetted from each tube. The final background salt gradient ranged from 1.075 g/ml to 1.168 g/ml, as determined by refractometry.

2.4. Gradient gel electrophoresis

Gradient gel electrophoresis, according to the method of Nichols et al (70), was performed on ultracentrifugal serum $d \leq 1.063$ and $d \leq 1.20$ g/ml fractions as well as on density gradient subfractions of LDL, $F^{o}_{1.20}9\text{-}28$ lipoproteins and HDL. The serum $d \leq 1.063$ fractions and density gradient subfractions of LDL and $F^{o}_{1.20}9\text{-}28$ lipoproteins were electrophoresed on 2-16% gradient gels (Pharmacia Fine Chemicals, Piscataway, NJ), while the $d \leq 1.20$ g/ml fractions and density gradient subfractions of HDL were electrophoresed on 4-30% gradient gels. Each gel was calibrated with high molecular weight protein standards (Pharmacia Fine Chemicals, Piscataway, NJ); latex beads of diameter 380±7.5A (Dow

Chemical, Indianapolis,IN) were also added to lanes containing the protein standard in the 2-16% gradient gels. Gels were stained for protein with Coomassie G-250 or for lipid with Oil Red O; standard and sample lanes were densitometrically scanned. Relative migration values (R_f values) for components on the electrophoretic pattern were calculated by taking the ratio of the migration distance of the component's peak relative to the migration distance of the peak of bovine serum albumin (4-30% gels) or apoferritin (2-16% gels) on the same gel. Approximate lipoprotein particle sizes were obtained from a calibration curve of migration distance vs Stokes' radii of protein standards run on each gel (45).

2.5. Electron Microscopy

Selected subfractions from density gradient ultracentrifugation of serum fractions from several baboons were dialyzed to 5 mM NH_4HCO_3 and, after negative staining with 1% sodium phosphotungstate (pH 7.4), examined with a JEM 100C electron microscope (JEOL Ltd., Tokyo, Japan). Particle size determinations were based on measurement of at least 200 particles within representative fields.

2.6. Lipid and Protein Assay

Assays for cholesterol and cholesteryl ester were performed on lipoprotein fractions using the colormetric methods of Roeschlau et al (74) (BMC Reagent Set No. 124087,

Boehringer Mannheim, Indianapolis, IN). Triglycerides were measured by a modification of the fully enzymatic method of Bucolo and David (75) (BMC Reagent Set No. 126012, Boehringer Mannheim, Indianapolis, IN); phospholipid phosphorus was measured by the method of Bartlett (76). Protein concentrations were measured by the method of Lowry et al (77), using bovine serum albumin (fatty acid-free fraction V) as a standard.

2.7. Serum and Lipoprotein Cholesterol Analyses

Cholesterol was measured in whole serum and in the supernatant after heparin-manganese chloride precipitation (79,80) by an enzymatic method using the ABA 100 Bichromatic Analyzer (Abbot Laboratories, South Pasadena, CA) (81). By convention, the cholesterol in the supernatant (non-precipitable cholesterol) was designated high density lipoprotein cholesterol (HDL-C). The difference between the total serum cholesterol and HDL-C was designated very low density and low density lipoprotein cholesterol (VLDL+LDL-C). The coefficient of variation for duplicate analyses was less than 2%.

Serum and non-precipitable cholesterol were measured in all five sires and a randomly selected subgroup of the progeny in Experiment I. These measurements were also performed on all 24 animals in Experiment II at 4, 5 and 6 weeks after the beginning of each diet period.

2.8. Apolipoprotein Analysis

Subfractions of the d 1.006-1.063 g/ml fractions were delipidated according to the method of Shore and Shore (73). sodium dodecyl sulfate-polyacrilamide electrophoresis was performed on 4% and 10% gels in 0.1% sodium dodecyl sulfate according to the method of Weber and Osborne (82). Molecular weights of protein bands were determined relative to the migration of a high molecular weight standard mixture (Pharmacia Fine Chemicals, Piscataway, NJ). Approximate weight percent of each protein was determined by measuring the area under the densitometric tracing after the gels were stained with Coomassie R-250. Apolipoproteins were identified by comparison with molecular weights for baboon apolipoproteins determined by Blaton and Peeters (8). These identifications were supported by apolipoprotein analyses using radioimmunoassay with antibodies raised against human apolipoproteins performed on selected ultracentrifugal fractions by Dr. John Albers, University of Washington School of Medicine, Seattle, Washington (83).

3. Statistical Methods

3.1. Statistical Methods - Experiment I

Lipoprotein concentrations were compared between families by the student's t-test. Correlations between concentrations of different lipoprotein classes or subpopulations were made by linear regression analysis. Lipoprotein concentrations of the group of animals consuming different diets (outlined in Table 3) were compared by the paired t-test between diets.

3.2. Statistical Methods - Experiment II

Analytic ultracentrifugal lipoprotein concentrations and lipoprotein cholesterol measurements made on the serum from each baboon after six weeks on each diet were transformed with a natural logarithm transformation in order to normalize the data, stabilize the variances and calculate the separate and combined effects of sex and diet (described below). When measured concentrations of zero were encountered, the transformation, logarithm(1+x) was used. Even after this transformation it was not possible to stabilize the variance for the concentration measurements of lipoproteins within the $F^{o}_{1.20}14\text{-}20$, $F^{o}_{1.20}20\text{-}28$ and $F^{o}_{1.20}20\text{-}28$ flotation intervals. These data were analyzed between diet groups and then for sex within diet groups by the non-parametric Mann-Whitney U-test.

All other data were analyzed by analysis of variance (84). The effects of time period, time period-by-sex, diet, diet-by-sex and carryover were tested against a within-animal error term (diet and sex mean square). The effects of carryover of the previous diets could not be estimated for analytic ultracentrifugal measurements made at density d 1.20 g/ml and, therefore, these carryover effects (if they exist) could not be excluded from the estimated diet effects. When the carryover effects from one diet to the next could be calculated, none were found to be statistically significant. However, the estimated means and diet effects were adjusted for the calculated carryover effects. The overall mean, sequence, sex and sequence-by-sex were tested against the animal within sequence and sex mean square. The estimated means (appropriate linear combinations of regression coefficients) and confidence intervals were based upon robust Huber (85) M-estimates (c=1.2) of the parameters. The linear model for analysis contained the terms for the overall mean, effect of diet, sex and diet-by-sex interaction.

The results are presented, in terms of group means, as the effects (60) of saturated fat in the presence of high cholesterol (the diet effect, F*HC), the sex effect (S) and the diet-by-sex interaction (FxS). The diet effect is defined as:

$$F*HC = (HCSF) - (HCPF).$$

The sex effect is defined as:

$$S = \{(HCSF) + (HCPF)\}_{male} - \{(HCSF) + (HCPF)\}_{female}.$$

The diet-by-sex interaction is given by one half the difference between the diet effect for males and females:

$$FxS = 1/2 \{(F*HC)_{male} - (F*HC)\}_{female}$$

and, therefore tells how much greater or lesser the diet effect is for males than for females.

The effects described above are differences on the logarithmic scale and, after application of the reverse transformation (exp(x)), represent the ratios of the original lipoprotein concentrations.

Chapter III: Lipoproteins in the Serum of Pedigreed Progeny Consuming the LARD+CS Diet - Experiment I

1. Experimental Design

Five adult male baboons were selected on the basis of either a hypercholesterolemic or hypocholesterolemic response to a diet high in saturated fat, in the form of lard, and cholesterol (the LARD+CS diet). These male baboons (sires) were each mated to female baboons (dams) which exhibited comparable serum cholesterol responses to the diet. The progeny resulting from these matings were the subjects of Experiment I.

In Experiment I, these pedigreed progeny consumed the LARD+CS diet from weaning until the time examined (between 12 and 56 months of age). The serum from these progeny were used for all studies described in Chapters III, IV and V of this dissertation. More information on these baboon progeny and on the design of Experiment I is provided in Chapter II, Sections 1.1 and 1.3.

2. High Density Lipoproteins (HDL)

2.1. Concentrations of High Density Lipoproteins in the Serum d<1.20 g/ml Fraction.

Figure 1 shows the analytic ultracentrifugal schlieren patterns of the 30 minute frame of the serum d≤1.20 g/ml fraction from two progeny in the study. All baboons examined exhibited lipoprotein material floating within the $F^{o}_{1.20}0\text{-}9$ interval. This flotation rate interval corresponds exactly with that previously observed for baboon HDL by Howard et al (23) and is similar to the flotation rate interval for HDL of other animal species, as well (71,86). The HDL, measured as material floating within the $F^{o}_{1.20}0\text{-}9$ interval, generally appeared as a broad peak of approximate flotation rate $F^{o}_{1.20}5.0$ (Figures 1a and 1b), but also exhibited differing amounts of additional components within the lower flotation rates (for example, the shoulder at approximately $F^{o}_{1.20}2.0$ in Figure 1b). In addition to HDL within the $F^{o}_{1.20}0\text{-}9$ flotation rate interval, many progeny in this study possessed measurable lipoprotein material floating within the $F^{o}_{1.20}9\text{-}28$ interval (Figure 1b). This lipoprotein material has not been previously reported in the baboon and is characterized in this dissertation.

The mean HDL concentration of all progeny in this study was 458 mg/dl (Table 5). The range of values of HDL concentration for all baboon progeny on the LARD+CS diet was from

Figure 1.

Analytic ultracentrifugal schlieren patterns and gradient gel (4-30%) electrophoretic patterns of the serum $d \leq 1.20$ g/ml fractions from two typical baboon progeny. These patterns illustrate major types of lipoprotein distributions observed in this study.

(a). Schlieren pattern from a baboon (animal 2592) demonstrating HDL (410 mg/dl) within the $F^{o}_{1.20}0\text{-}9$ flotation rate range, and essentially no material (4 mg/dl) in the $F^{o}_{1.20}9\text{-}28$ interval. LDL ($F^{o}_{1.20}28\text{-}56$) does not appear in the frame shown.

(b). Schlieren pattern from a baboon (animal 1816) exhibiting both HDL (510 mg/dl, within the $F^{o}_{1.20}0\text{-}9$ flotation interval) and $F^{o}_{1.20}9\text{-}28$ lipoprotein (207 mg/dl).

(c). Gradient gel electrophoretic pattern for baboon 2592 possessing a polydisperse distribution of HDL, primarily within the 72-125A particle size range. LDL appears as material of size greater than 220A.

(d). Gradient gel electrophoretic pattern for baboon 1816 exhibiting a polydisperse distribution of HDL material within the 72-125A particle size range and $F^{o}_{1.20}9\text{-}28$ lipoproteins within the 125-220A particle size range.

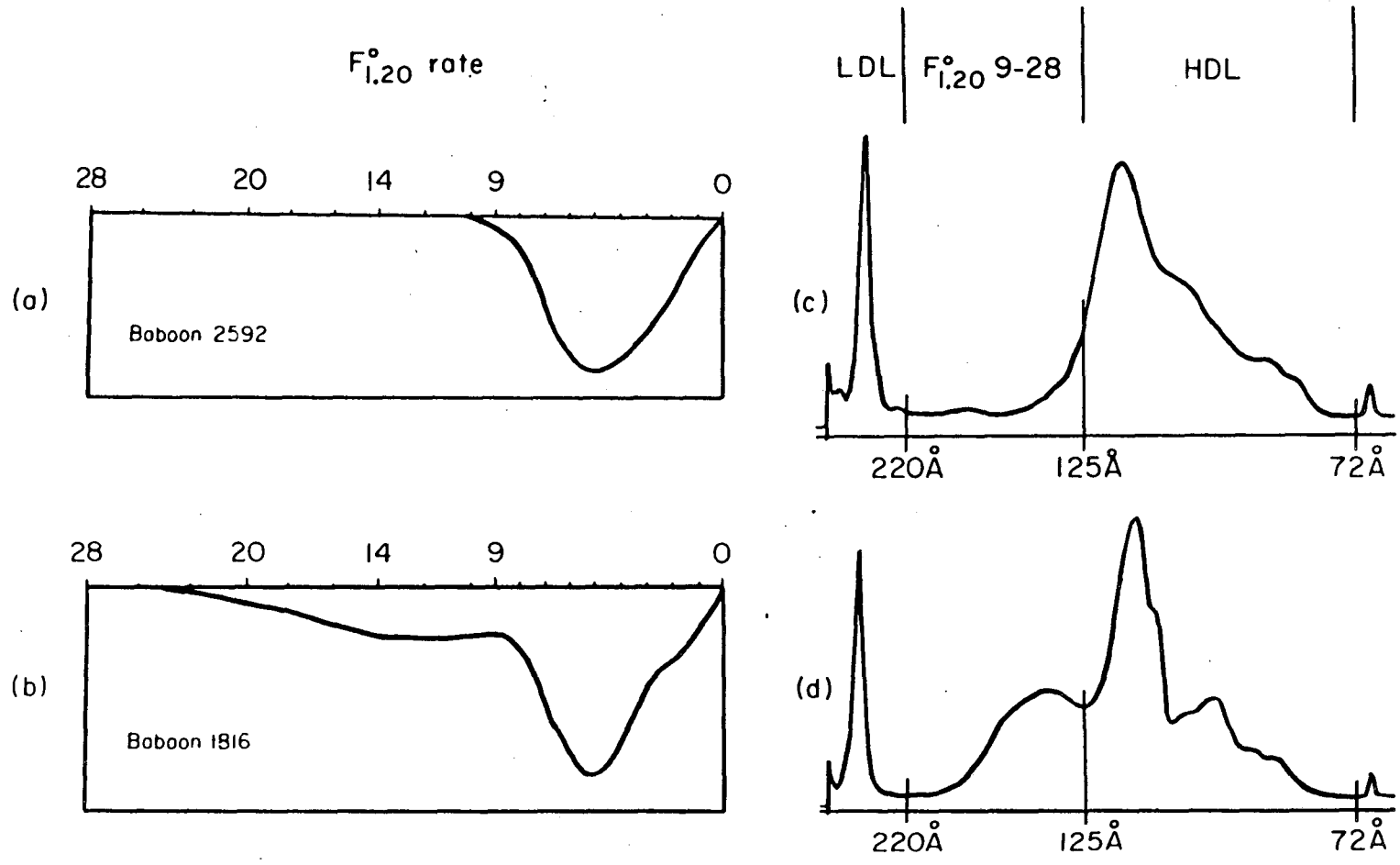

$F^{\circ}_{1.20}$ rate
LDL
$F^{\circ}_{1.20}$ 9-28
HDL
28
20
14
9
0
(a)
Baboon 2592
(b)
Baboon 1816
(c)
(d)
220Å
125Å
72Å

Table 5. Mean ± SD serum concentrations (mg/dl) of HDL and $F^{o}_{1.20}9\text{-}28$ lipoproteins and peak $F^{o}_{1.20}$ rate (svedbergs) (within the serum d≤1.20 g/ml fraction) for all pedigreed progeny studied in Experiment I (n=46).

Lipoprotein Species	Group Mean ± SD	Range of Values
HDL ($F^{o}_{1.20}0\text{-}9$)	466 ± 78	310 - 660
Peak $F^{o}_{1.20}$ Rate	4.99 ± .30	3.68 - 5.56
HDL-I	291 ± 91	118 - 466
HDL-II	102 ± 32	56 - 193
HDL-III-V	73 ± 16	37 - 112
$F^{o}_{1.20}9\text{-}28$	108 ± 98	1 - 410

310 to 660 mg/dl. The peak flotation rate of the HDL was $F^{o}_{1.20}4.99 \pm 0.30$ (mean±SD) with a range of observed values of $F^{o}_{1.20}3.68$ to $F^{o}_{1.20}5.56$.

2.2. Characterization of HDL Size Distributions by Gradient Gel Electrophoresis

The particle size distribution of HDL material in the serum $d \leq 1.20$ g/ml fraction of all progeny was examined by gradient gel electrophoresis. As demonstrated in Figures 1a and 1c, progeny which exhibited only HDL material by analytic ultracentrifugation possessed lipoprotein material by gradient gel electrophoresis within the 72-125A particle size range. Progeny which demonstrated HDL and $F^{o}_{1.20}9\text{-}28$ lipoprotein material by analytic ultracentrifugation (Figure 1b) had gradient gel electrophoresis patterns that included HDL material within the 72-125A particle size range and additional material ($F^{o}_{1.20}9\text{-}28$ lipoproteins) within the 125-220A particle size range (Figure 1d).

Gradient gel electrophoretic patterns of HDL in the $d \leq 1.20$ g/ml fraction from all progeny studied, irrespective of level of $F^{o}_{1.20}9\text{-}28$ lipoproteins, showed a multicomponent distribution in the 72-125A particle size range. This material could be isolated within the d 1.063-1.20 g/ml interval and was comparable to human HDL in both size and density range (45). The major part of baboon HDL was contained within a single peak corresponding to particles in the 100-125A size range. The remaining material was

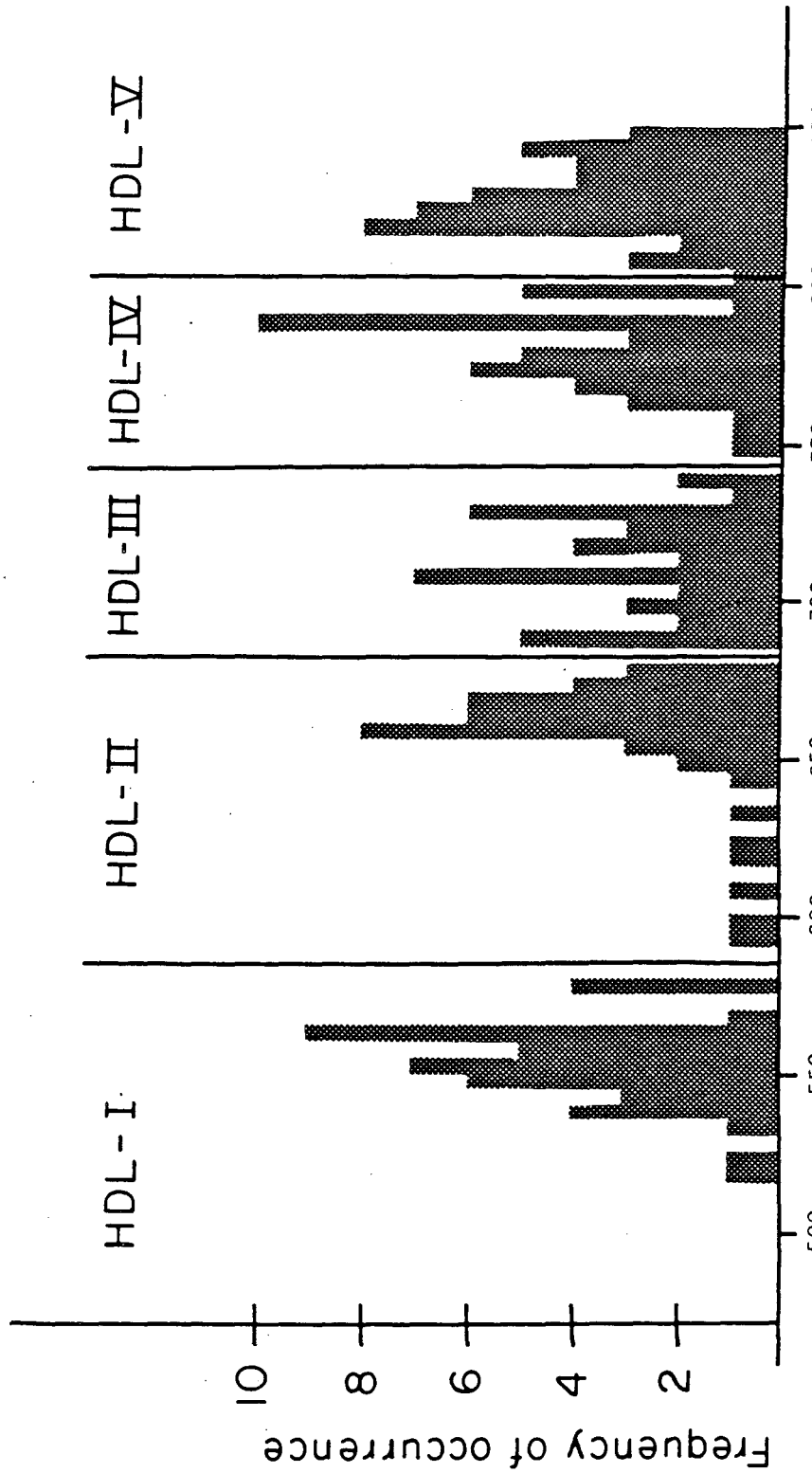
distributed among 3-4 peaks in the size range of 72-100A. When the frequency of observation of individual peaks in 42 gradient gel electrophoresis patterns from individual animals was plotted versus each peak's R_f value (see Materials and Methods), distinct frequency maxima (Figure 2) occurred within the specific size intervals. The R_f intervals containing the frequency maxima were used to define baboon HDL subpopulations (designated HDL-I through HDL-V). No significant differences were observed in R_f intervals or R_f values at the frequency maxima of HDL subpopulations when patterns were compared on the basis of serum levels of $F^{o}_{1.20}9\text{-}28$ lipoproteins.

2.3. Physical-Chemical Properties of HDL Subpopulations

The physical-chemical properties of baboon HDL subpopulations in the sera of two progeny (who possessed $F^{o}_{1.20}9\text{-}28$ lipoprotein levels of 171 mg/dl and 207 mg/dl) were studied after subfractionation by equilibrium density gradient ultracentrifugation of the d 1.063-1.20 g/ml fraction. Figure 3 shows the gradient gel electrophoretic patterns of the whole HDL fraction and fourteen density subfractions isolated from the serum of one of the progeny. Most subfractions were monodisperse when analyzed by gradient gel electrophoresis, though polydispersity was apparent in subfractions of higher hydrated density. The mean size (determined from migration of the peak) of lipoproteins in each density subfraction was used to assign the lipoproteins to specific

Figure 2.

Frequency of occurrence of R_f values of HDL peaks as observed on 4-30% electrophoretic gradient gels. Electrophoresis was performed on the serum $d \leq 1.20$ g/ml fraction from 42 baboon progeny from all five families studied. These progeny exhibited the full range of $F^{o}_{1.20}$ 9-28 lipoprotein concentrations. The five baboon HDL subpopulations determined from this frequency plot are indicated (HDL-I through HDL-V).

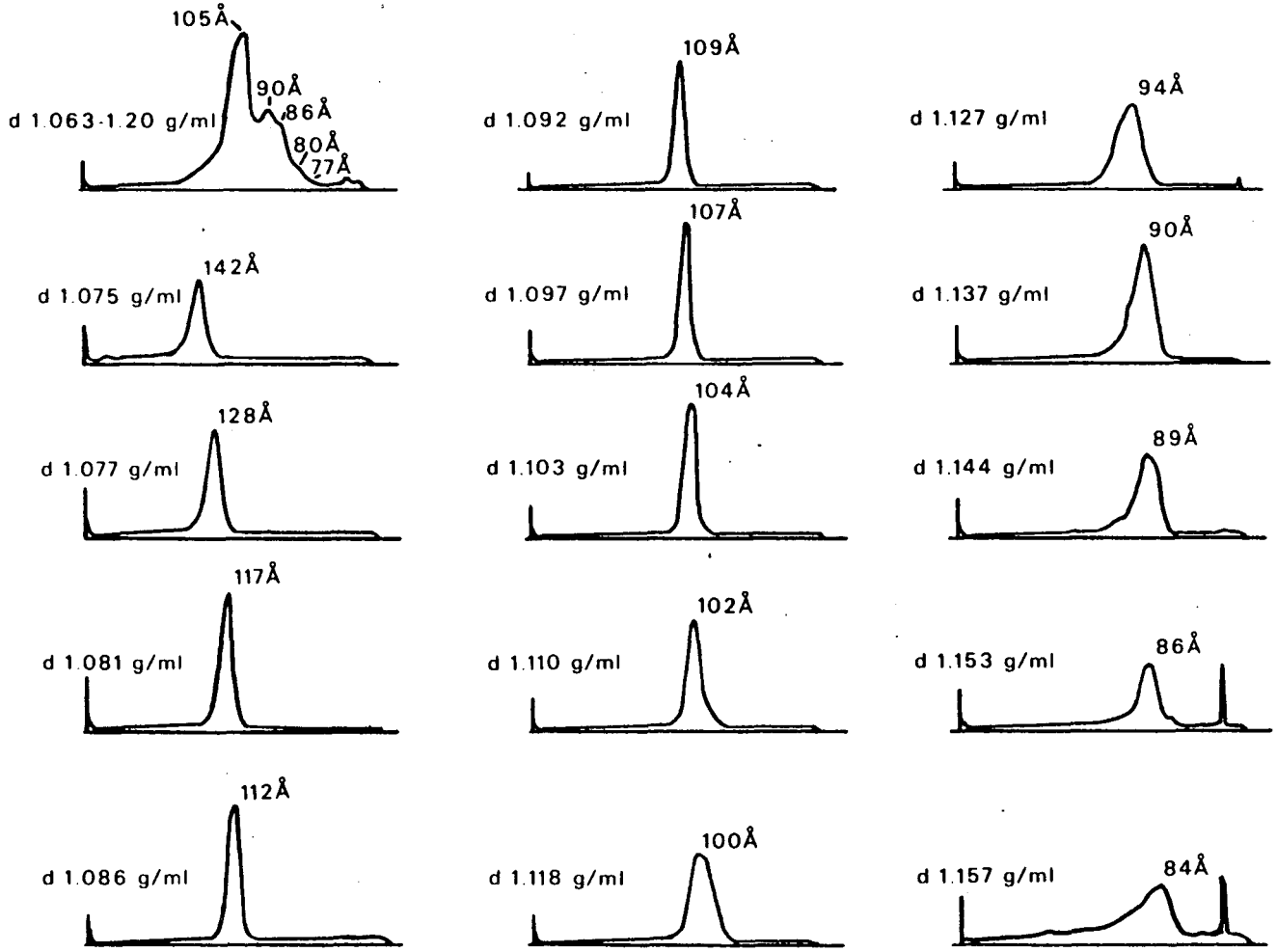

HDL-I
HDL-II
HDL-III
HDL-IV
HDL-V
Frequency of occurrence
2
4
6
8
10
500
550
600
650
700
750
800
850
Rf Value

XBL832-3622

Figure 3.

Gradient gel electrophoretic patterns (4-30% gels) of density subfractions of the d 1.063-1.20 g.ml fraction from baboon 2033. These patterns illustrate the trend of decreasing particle size with increasing hydrated density and the general observation that the HDL material in least dense subfractions was monodisperse by gradient gel electrophoresis.

HDL DENSITY SUBFRACTIONS



HDL subpopulations (HDL-I, HDL-II, etc.); the particle size ranges of the HDL subpopulations were previously defined on the basis of the frequency histogram (Figure 2). Based on the relationship between the mean particle size and hydrated density of the subfractions, it was possible to assign hydrated density ranges for particles within each HDL subpopulation. Density subfractions which best represented individual HDL subpopulations and showed minimal contamination from other subpopulations were analyzed for chemical composition and apolipoprotein content. The R_f intervals, the corresponding particle size and hydrated density ranges of baboon subpopulations HDL-I through HDL-V are given in Table 6. The particle size of the HDL subpopulations decreased regularly (100-125A, HDL-I through 72-78A, HDL-V) with increasing hydrated density (1.063-1.20 g/ml, HDL-I through 1.160-1.120 g/ml, HDL-V). Likewise, the chemical composition of HDL subpopulations varied with hydrated density, with the more dense fractions exhibiting a higher percent protein content (32.8%, HDL-I vs 57.6%, HDL-IV) and a lower percent phospholipid (37.6%, HDL-I vs 24.4%, HDL-IV) and cholesteryl ester (23%, HDL-I vs 12.8%, HDL-IV) content (see Table 6).

2.4. Apolipoprotein Content of HDL Subpopulations

The apolipoprotein composition of density gradient subfractions of HDL was examined by sodium dodecyl sulfate-polyacrilamide electrophoresis and gave results very similar to the observations reported by Blaton and Peeters (8) for

Table 6. Properties of the major HDL in the serum of Baboon B in Tables 8 and 11.

	HDL subpopulation				
	I	II	III	IV	V
R_f Range	.460-.585	.585-.685	.685-.745	.745-.805	.805-.900
Hydrated Density Range (g/ml)	1.063-1.120	1.120-1.135	1.135-1.150	1.150-1.200	
Approximate Size Range (A)	100-125	88-100	82-88	78-82	72-78
Composition (wt. %)					
Protein	32.8	47.5	54.8	57.6	ND
Phospholipid	37.6	31.1	27.6	24.4	ND
Cholesteryl Ester	23.0	18.1	14.6	12.8	ND
Unesterified Cholesterol	4.2	2.8	2.8	5.0	ND
Triglyceride	2.4	0.5	0.2	0.2	ND
Calculated $F^{o}_{1.20}$ Rate (svedbergs)	5.17	2.77	1.90	1.37	ND

ND: Not done

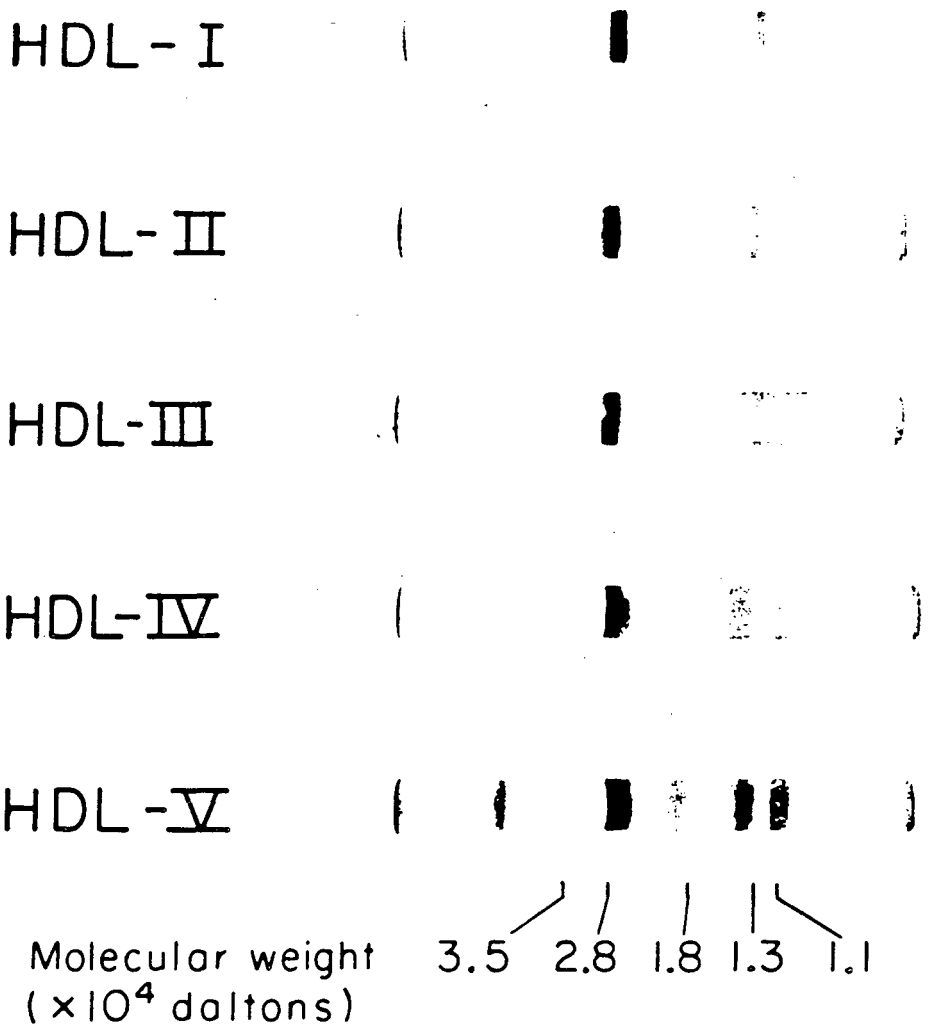
baboon total HDL. ApoA-I (2.8 x 10 daltons) was the major protein (>65% of total Coomassie staining material) in all subfractions, and all subfractions contained the same small molecular weight proteins (1.8 x 10^4, 1.3 x 10^4, and 1.1 x 10^4 daltons), including a small protein of 1.4-1.5 x 10^4 daltons which appeared as a shoulder on the peak of the 1.3 x 10^4 dalton protein (Figure 4). An additional protein of approximately 2.4 x 10^4 daltons was detected in all HDL subfractions, except the HDL-I subfraction, and was the same as a B-mercaptoethanol-reducible protein observed by Blaton and Peeters (8) in baboon HDL. No apoE (3.5 x 10^4 daltons) was apparent in any HDL subfractions.

2.5. Concentrations of Baboon HDL Subpopulations

Using the physical data for baboon HDL subpopulations given in Table 6, approximate $F^{o}_{1.20}$ rates for each subpopulation were calculated and concentrations of individual HDL subpopulations were estimated from the serum $d \leq 1.20$ g/ml schlieren pattern of each progeny. Table 5 breaks down the total HDL ($F^{o}_{1.20}$0-9) concentration into HDL subpopulations designated HDL-I, HDL-II, and the sum of HDL-III through HDL-V. The bulk (63%) of the HDL material was found, on average, as HDL-I. This was consistent with observations by gradient gel electrophoresis (see Figure 1c and 1d, for examples) which also demonstrated that the major HDL subpopulation was HDL-I (major HDL peak within the 100-125A particle size range). HDL-II and HDL-III through HDL-V

Figure 4.

Sodium dodecyl sulfate-polyacrilamide electrophoretic gels of selected density subfractions of baboon HDL, showing the apolipoprotein composition of baboon HDL subpopulations HDL-I, HDL-II, HDL-III, HDL-IV and HDL-V.


HDL-I
HDL-II
HDL-III
HDL-IV
HDL-V
Molecular weight
(×10⁴ daltons)
3.5
2.8
1.8
1.3
1.1

constituted, on average, 22% and 16% of the HDL material, respectively. The predominance of the HDL-I species with an $F^{o}_{1.20}$ rate of 5.17 (from Table 6) accounted for the observation of a peak $F^{o}_{1.20}$ rate close to $F^{o}_{1.20}5.0$ for whole HDL (Table 5).

2.6. Statistical Correlations among Concentrations of HDL Subpopulations

Possible metabolic or physical relationships among HDL subpopulations were examined by calculating statistical correlation coefficients among concentrations of baboon HDL subpopulations, and with the total HDL concentration and peak flotation ($F^{o}_{1.20}$) rate (Table 7). Total HDL concentrations correlated positively with both HDL-I and HDL-II concentrations ($r = .90$ and $r = .44$, respectively), but not at all with HDL-III through HDL-V concentrations. The HDL peak $F^{o}_{1.20}$ rate depended inversely upon HDL-II concentrations ($r = -.44$), while HDL-I and HDL-III through HDL-V concentrations were also inversely related ($r = -.43$).

Table 7. Correlation coefficients among the concentrations of lipoprotein species within the d≤1.20 g/ml serum fraction from all the progeny studied in Experiment I (n=46).

	HDL-I	HDL-II	HDL-III-V	Peak $F^{o}_{1.20}$ Rate
Total HDL	.89[a]	.44[a]	.00	-.03
HDL-I	-	.04	-.43[a]	.23[b]
HDL-II		-	.22[b]	.42[a]
HDL-III-V			-	-.19

a: P<0.005

b: P<0.1

3. $F^{o}_{1.20}9\text{-}28$ Lipoproteins

3.1. Flotation and Size Heterogeneity of $F^{o}_{1.20}9\text{-}28$ Lipoproteins

The total lipoprotein $d \leq 1.20$ g/ml fraction isolated from the serum of progeny of all sires was examined by analytic ultracentrifugation and gradient gel electrophoresis. Ultracentrifugal patterns for this fraction from all progeny demonstrated HDL material within the $F^{o}_{1.20}0\text{-}9$ flotation interval and LDL material within the $F^{o}_{1.20}28\text{-}56$ flotation interval. Electrophoretic patterns of lipoproteins in the $d \leq 1.20$ g/ml fraction indicated the presence of peaks corresponding to HDL and LDL within the particle size ranges of 72-125A and 220-290A, respectively. In contrast, not all progeny possessed measurable lipoprotein material within the $F^{o}_{1.20}9\text{-}28$ flotation interval. $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations measured by analytic ultracentrifugation in the serum of progeny examined ranged from 1 to 410 mg/dl with an overall mean$\pm$SD of 108$\pm$98 mg/dl (Table 5). When analytic ultracentrifugation patterns demonstrated measurable levels of $F^{o}_{1.20}9\text{-}28$ lipoproteins, the gradient gel electrophoretic patterns exhibited an additional peak within the 125-220A particle size range. The extent of heterogeneity of flotation properties and particle size of these additional species depended on the total concentration of $F^{o}_{1.20}9\text{-}28$ lipoproteins.

Figure 5 illustrates the relationships between flotation rate heterogeneity and total $F^{o}_{1.20}9\text{-}28$ concentration by plotting the concentrations of material within the $F^{o}_{1.20}9\text{-}14$, $F^{o}_{1.20}14\text{-}20$ and $F^{o}_{1.20}20\text{-}28$ subintervals versus the total $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration. In general, when a baboon possessed a serum $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration of less than 70 mg/dl, almost all of this material was found by analytic ultracentrifugation to occur within the $F^{o}_{1.20}9\text{-}14$ flotation interval and to extend from the HDL interval (defined by the flotation rate range of $F^{o}_{1.20}0\text{-}9$). When baboons exhibited serum levels between 70 and 110 mg/dl of $F^{o}_{1.20}9\text{-}28$ lipoproteins, these lipoproteins were observed as a continuum of material extending from $F^{o}_{1.20}9$ up to $F^{o}_{1.20}20$. Only baboons which possessed $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations above 110 mg/dl were found to exhibit lipoprotein material floating beyond $F^{o}_{1.20}20$; but in all cases the $F^{o}_{1.20}9\text{-}28$ lipoprotein material extended from the upper flotation rate limit ($F^{o}_{1.20}9$) of the HDL distribution.

Representative examples of the relationship between concentration vs flotation rate and particle size of the $F^{o}_{1.20}9\text{-}28$ lipoproteins are illustrated in Figure 6 which shows computer-derived ultracentrifugal schlieren patterns (30 min frame following attainment of 52,640 rpm) and electrophoretic patterns (4-30% gradient gel) of the total lipoprotein $d \leq 1.20$ g/ml fraction isolated from the sera of four progeny that exhibited a wide range of $F^{o}_{1.20}9\text{-}28$

Figure 5.

Relationship between flotation rate heterogeneity of $F^{o}_{1.20}9\text{-}28$ lipoproteins and the total concentration of $F^{o}_{1.20}9\text{-}28$ lipoproteins. The graphs demonstrate that, for $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations below approximately 70 mg/dl, almost all this material is found within the $F^{o}_{1.20}9\text{-}14$ flotation interval. As $F^{o}_{1.20}9\text{-}28$ concentrations increased to about 110 mg/dl, material was distributed up to $F^{o}_{1.20}20$. When a baboon exhibited $F^{o}_{1.20}9\text{-}28$ lipoprotein levels above 110 mg/dl, this material appeared throughout the entire $F^{o}_{1.20}9\text{-}28$ interval.

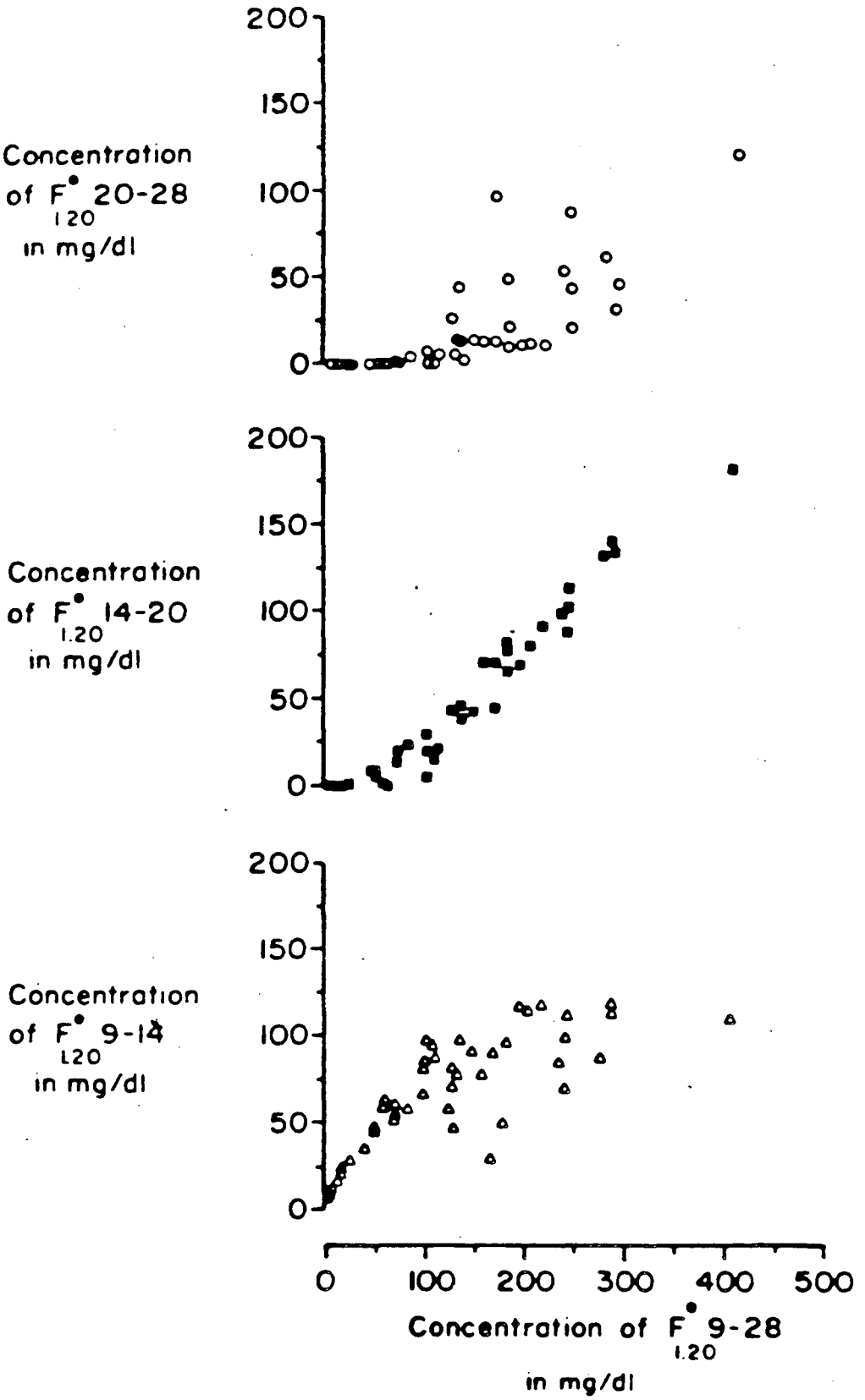

Concentration of F* 20-28 in mg/dl
Concentration of F* 14-20 in mg/dl
Concentration of F* 9-14 in mg/dl
200
150
100
50
0
0
100
200
300
400
500
Concentration of F* 9-28 in mg/dl

Figure 6.

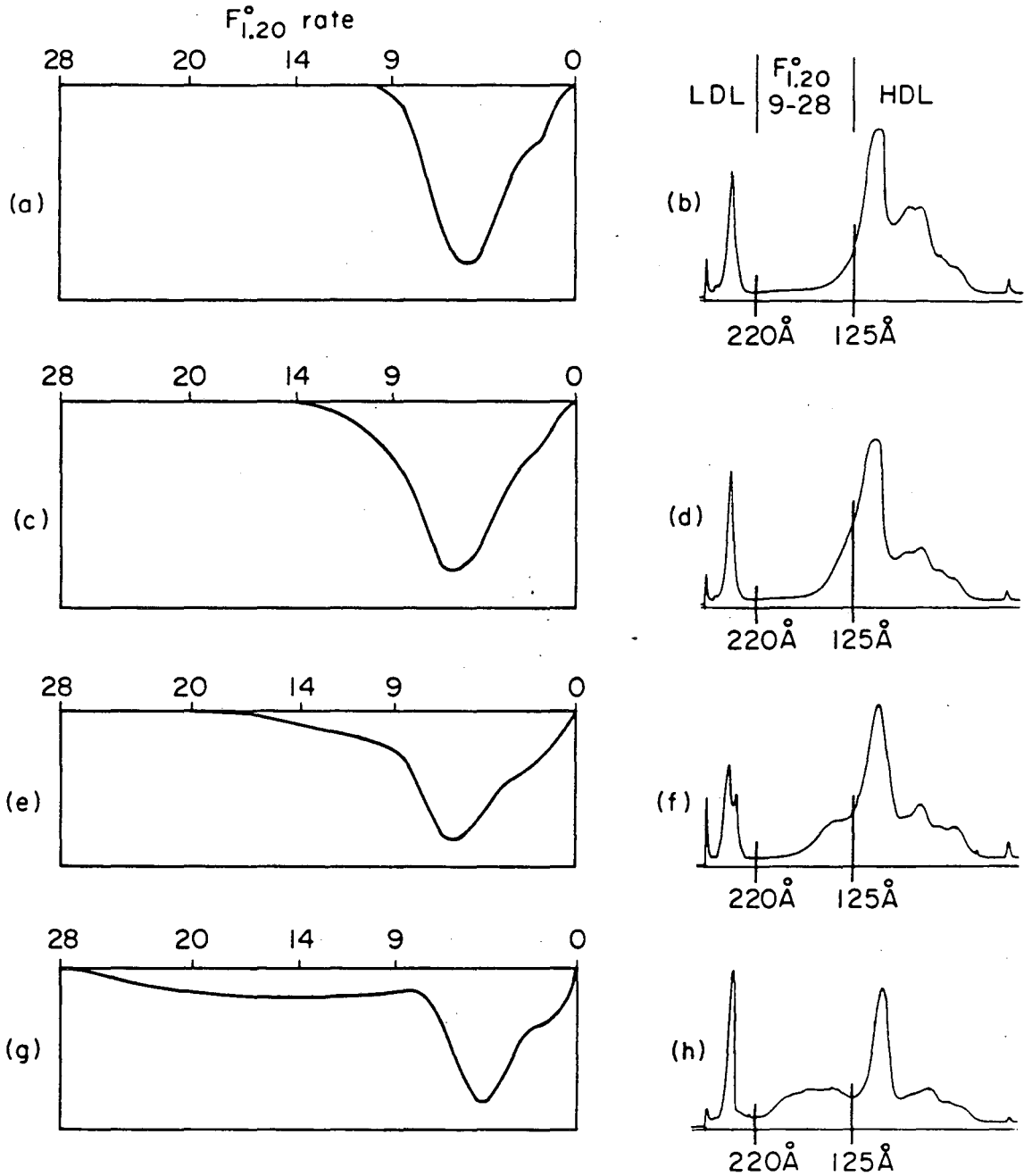
Computer-derived analytic ultracentrifugal schlieren patterns (a,c,e,g) and 4-30% gradient gel electrophoretic patterns (b,d,f,h) of the serum $d \leq 1.20$ g/ml fraction from four baboons possessing different concentrations of $F^{o}_{1.20}9\text{-}28$ lipoproteins.

(a). Schlieren pattern of a baboon with essentially no $F^{o}_{1.20}9\text{-}28$ material (9mg/dl). HDL floats within the $F^{o}_{1.20}0\text{-}9$ flotation interval (LDL not shown in this frame).

(b). Gradient gel electrophoretic pattern of the serum $d \leq 1.20$ g/ml fraction of this animal showing a multicomponent HDL profile predominantly within the 72-125A size range. LDL and IDL appear in size range 220-290A.

(c) and (d). Ultracentrifugal and electrophoretic patterns, respectively, of the serum $d \leq 1.20$ g/ml fraction from a baboon with an $F^{o}_{1.20}9\text{-}20$ concentration of 65 mg/dl. The $F^{o}_{1.20}9\text{-}28$ material is confined to the $F^{o}_{1.20}9\text{-}14$ flotation interval (c) and appears as a shoulder on the trailing edge on the HDL gradient gel electrophoretic profile (d).

(e), (f), and (g), (h). Patterns of the serum $d \leq 1.20$ g/ml fractions from two baboons with progressively increasing $F^{o}_{1.20}9\text{-}28$ concentrations (104 mg/dl and 238 mg/dl, respectively). With increasing $F^{o}_{1.20}9\text{-}28$ concentration, the schlieren pattern includes a wider range of material within the $F^{o}_{1.20}9\text{-}28$ interval ($F^{o}_{1.20}9\text{-}20$ in (e) and $F^{o}_{1.20}9\text{-}28$ in (g)); the gradient gel electrophoretic pattern shows material extending considerably beyond the upper limit of the HDL profile (125-190A in (f) and 125-220A in (h)) with increasing total $F^{o}_{1.20}9\text{-}28$ concentration.


$F^{\circ}_{1.20}$ rate
28
20
14
9
0
(a)
(b)
LDL
$F^{\circ}_{1.20}$ 9-28
HDL
220Å
125Å
(c)
(d)
(e)
(f)
(g)
(h)

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lipoprotein concentrations (from 9 to 238 mg/dl). At negligible $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations (9 mg/dl; schlieren pattern shown in Figure 6a), essentially all lipoprotein material detected in this frame was confined to the $F^{o}_{1.20}$ 0-9 flotation interval and correspond to HDL normally occurring in baboon serum. LDL floating within the $F^{o}_{1.20}28\text{-}56$ interval did not appear in this frame. The corresponding electrophoretic pattern of HDL in this fraction (Figure 6b) showed peaks mainly in the 72 to 125A size range. LDL peaks appeared at the far left in these scans, and were not well resolved on 4-30% gradient gels. At a $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration of 65 mg/dl (Figure 6c), the schlieren pattern again showed the major part of HDL within the $F^{o}_{1.20}0\text{-}9$ interval, but some additional material could now be seen extending into the $F^{o}_{1.20}9\text{-}14$ flotation rate interval. The gradient gel electrophoretic pattern for this fraction (Figure 6d) exhibited a definite shoulder on the major peak of the HDL, also indicating the presence of additional lipoprotein material which ranged in size up to 165A. At a total $F^{o}_{1.20}9\text{-}28$ concentration of 104 mg/dl (Figure 6e), the schlieren pattern area beyond $F^{o}_{1.20}0\text{-}9$ now included flotation rates up to $F^{o}_{1.20}20$. The corresponding gradient gel electrophoretic pattern (Figure 6f) showed a broad peak between the HDL and LDL profiles. While the mean particle size of this additional material was 140A, its overall size range extended to 190A. Figure 6g and 6h show the schlieren and electrophoretic patterns,

respectively, of the total lipoprotein d≤1.20 g/ml fraction isolated from the serum of a progeny that had an $F^{o}_{1.20}9\text{-}28$ concentration of 238 mg/dl. The schlieren pattern now included material with flotation rates within the entire $F^{o}_{1.20}9\text{-}28$ range. The gradient gel electrophoretic pattern exhibited a broad profile beyond the HDL indicative of heterogeneous material ranging in size from 125 to 220A.

3.2. $F^{o}_{1.20}9\text{-}28$ Lipoproteins in the Serum d≤1.063 g/ml Fraction

The d≤1.063 g/ml fraction isolated from the serum of all progeny was also examined by analytic ultracentrifugation and gradient gel electrophoresis. In general, ultracentrifugal patterns of lipoproteins in the d≤1.063 g/ml fraction from sera of baboons who possessed $F^{o}_{1.20}9\text{-}28$ lipoproteins showed a distinct peak within the $S^{o}_{f}0\text{-}5$ flotation interval. Gradient gel electrophoretic patterns (2-16% gel) also showed a separate peak within the 125-220A size range consistent with presence of $F^{o}_{1.20}9\text{-}28$ lipoproteins in this fraction. The concentration of material within the $S^{o}_{f}0\text{-}5$ interval was highly comparable to the concentration of $F^{o}_{1.20}9\text{-}28$ lipoproteins ($S^{o}_{f}0\text{-}5$ vs $F^{o}_{1.20}9\text{-}28$, Pearson r = .93, $P < 0.01$; slope = .99, intercept = -11.4 mg/dl), indicating that most, but not all, of the $F^{o}_{1.20}9\text{-}28$ lipoprotein material was present in the d≤1.063 g/ml fraction. $F^{o}_{1.20}9\text{-}28$ lipoprotein material observed in the serum d 1.063-1.20 g/ml fraction was confined to the d 1.063-1.080

g/ml portion of this fraction (see Figure 3).

Further evidence demonstrating the identity of material within the $S^{o}_{f}0\text{-}5$ flotation interval (analytic ultracentrifugation run at d 1.063 g/ml) and material within the $F^{o}_{1.20}9\text{-}28$ flotation interval (analytic ultracentrifugation run at d 1.20 g/ml) was obtained by performing analytic ultracentrifugation of the serum $d \leq 1.063$g/ml fraction at a density of d 1.20 g/ml. Figure 7 shows the analytic ultracentrifugation schlieren patterns obtained for lipoprotein species in the serum $d \leq 1.063$ g/ml fraction (Figure 7a) and in the $d \leq 1.20$ g/ml serum fraction (Figure 7b). The concentration of lipoproteins within the $S^{o}_{f}0\text{-}7$ flotation interval in Figure 7a was measured as 258 mg/dl (The $S^{o}_{f}0\text{-}7$ flotation interval was used instead of the $S^{o}_{f}0\text{-}5$ flotation interval because $S^{o}_{f}7$ is the location of the minimum between the two major peaks present in the schlieren pattern). The $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration measured in the schlieren pattern in Figure 7b was 278 mg/dl. Following adjustment of the background salt density of the serum $d \leq 1.063$ g/ml fraction to d 1.20 g/ml, this fraction was then subjected to analytic ultracentrifugation to evaluate its content of $F^{o}_{1.20}9\text{-}28$ lipoprotein material (Figure 7c). The concentration of $F^{o}_{1.20}9\text{-}28$ lipoprotein material obtained was 246 mg/dl which compares well with the measured amount of $S^{o}_{f}0\text{-}5$ lipoprotein material within this same fraction (258 mg/dl) and the amount of $F^{o}_{1.20}9\text{-}28$ lipoprotein material measured within the serum $d \leq 1.20$ g/ml fraction. It

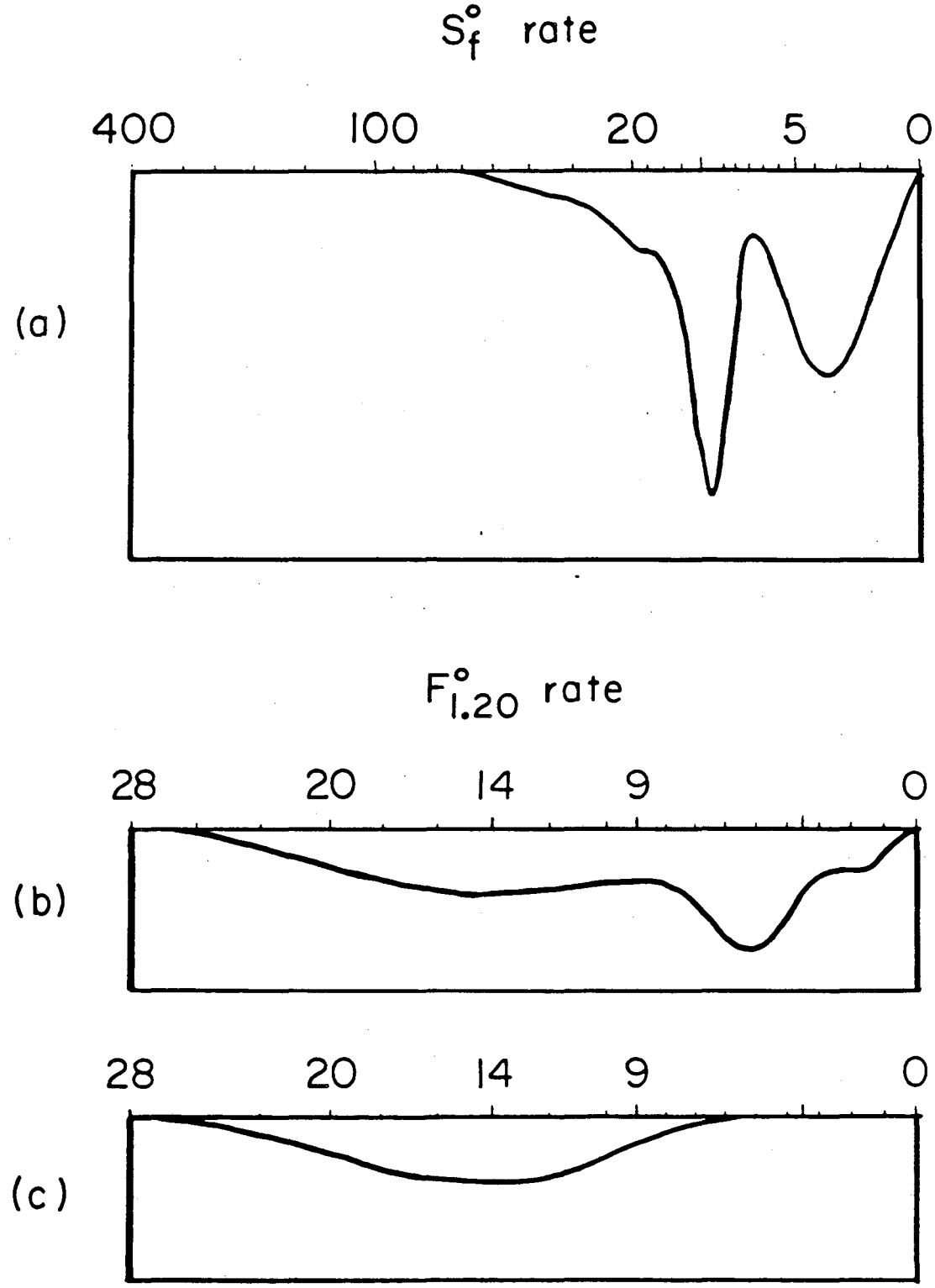
Figure 7.

Demonstration of the equivalence of material measured within the $S^{o}_{f}0-5$ flotation rate interval within the serum $d \leq 1.063$ g/ml fraction and the material measured within the $F^{o}_{1.20}9-28$ flotation rate interval within the serum $d \leq 1.20$ g/ml fraction.

(a). Schlieren pattern of the $d \leq 1.063$ g/ml fraction from the serum of one baboon in the study, possessing 258 mg/dl of lipoprotein material within the $S^{o}_{f}0-7$ flotation rate interval.

(b). Schlieren pattern of the $d \leq 1.20$ g/ml fraction from the serum of the same baboon exhibiting 278 mg/dl of lipoprotein material within the $F^{o}_{1.20}9-28$ flotation rate interval.

(c). Schlieren pattern of the $d \leq 1.063$ g/ml fraction from the same baboon after dialysis to d 1.20 g/ml. In this case, the concentration of $F^{o}_{1.20}9-28$ lipoprotein material was 246 mg/dl, comparable with the values in (a) and (b) above.


S_f° rate
400
100
20
5
0
(a)
$F_{1.20}^\circ$ rate
28
20
14
9
0
(b)
28
20
14
9
0
(c)

should be noted that the schlieren pattern in Figure 7c exhibited a broader range of flotation rate ($F^{o}_{1.20}6\text{-}28$) than the $F^{o}_{1.20}9\text{-}28$ interval.

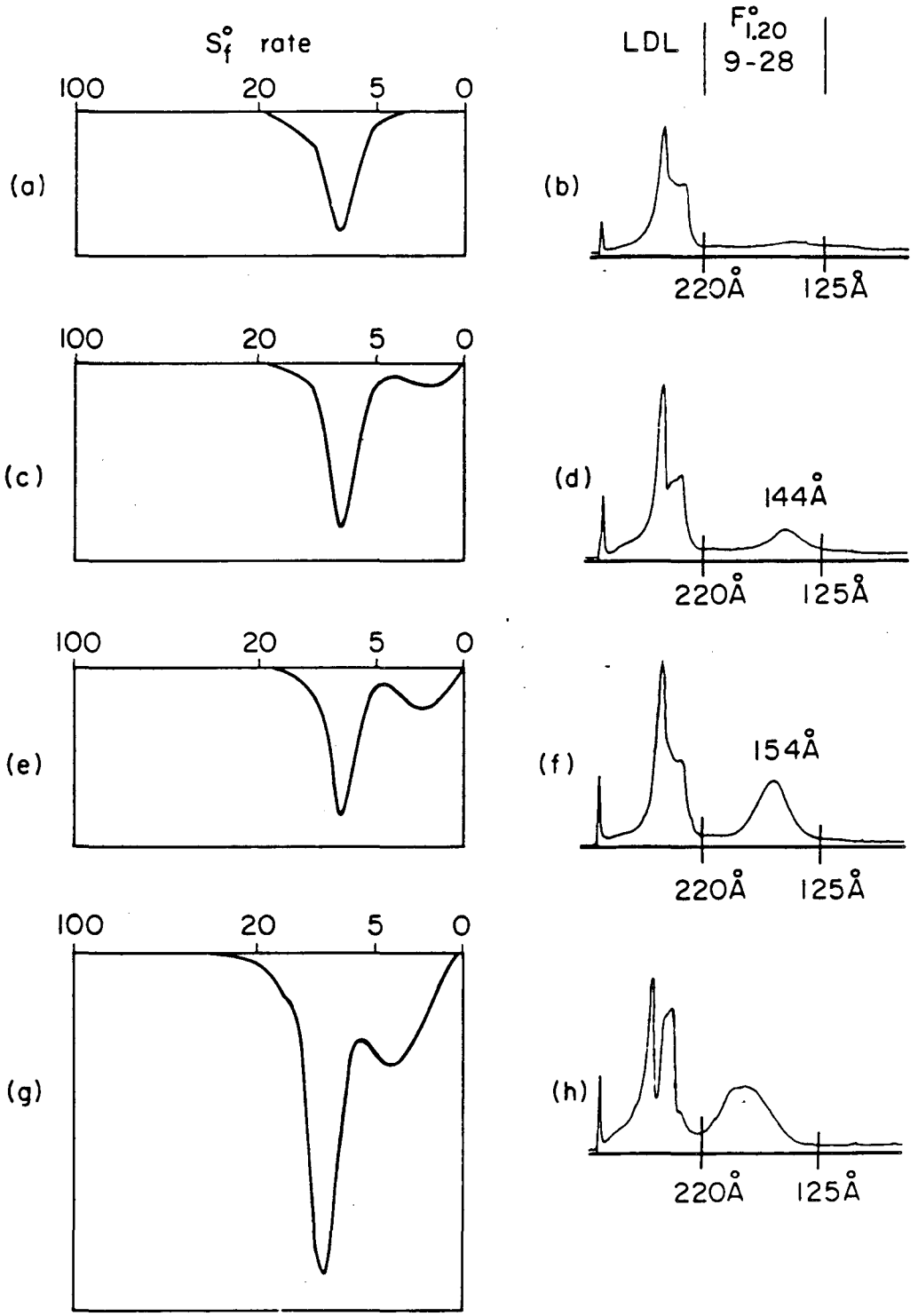
Figure 8 shows analytic ultracentrifugation schlieren and electrophoretic patterns (2-16% gels) of the $d \leq 1.063$ g/ml fraction isolated from the serum of the same four progeny described in Figure 6. As the serum $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration increased, there was a corresponding increase in the amount of material within the $S^{o}_{f}0\text{-}5$ flotation interval (Figure 8a, c, e, g). When these same fractions were examined by gradient gel electrophoresis, the electrophoretic patterns showed a progressive increase in amount and mean size of material in the particle size range of 125-220A with increasing total $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration (Figure 8b; 8d: mean size, 144A; 8f: mean size, 154A; 8h: heterogeneous material up to 220A). Since HDL were ultracentrifugally removed from these fractions, it is apparent that the $F^{o}_{1.20}9\text{-}28$ lipoproteins comprise essentially all the material observed within the 125-220A size range.

3.3. Subfractionation by Hydrated Density of $F^{o}_{1.20}9\text{-}28$ Lipoproteins Localized within the d1.006-1.063 g/ml Fraction

The d 1.006-1.063 g/ml fraction from each of five progeny was subjected to equilibrium density gradient ultracentrifugation in an attempt to isolate $F^{o}_{1.20}9\text{-}28$ lipoprotein material for further characterization. Even though the

Figure 8.

Computer-derived analytic ultracentrifugal schlieren patterns (a,c,e,g) and 2-16% gradient gel electrophoretic patterns (b,d,f,h) of the serum $d \leq 1.063$ g/ml fractions from the same four baboons described in Figure 6, possessing $F^{o}_{1.20}9\text{-}28$ concentrations of 9mg/dl, 65mg/dl, 104mg/dl, and 238 mg/dl, respectively. The schlieren pattern of the first baboon (a) shows the presence of only LDL ($S^{o}_{f}5\text{-}12$) and IDL ($S^{o}_{f}12\text{-}20$). Similarly, the gradient gel pattern (b) of the serum $d \leq 1.063$ g/ml fraction of this baboon shows only LDL and IDL material in the approximate size range 220-340A. The schlieren patterns of this fraction from the other baboons (c,e,g) show increasing levels of material within the $S^{o}_{f}0\text{-}5$ flotation interval which corresponds to the material that floats within the $F^{o}_{1.20}9\text{-}28$ interval (see text). Likewise, with increasing $F^{o}_{1.20}9\text{-}28$ concentrations, the gradient gel patterns (d,f,h) show progressively more material within the particle size range (125-220A) previously defined (see Figure 1) for the $F^{o}_{1.20}9\text{-}28$ material.


S_f° rate
100
20
5
0
(a)
(b)
LDL
$F^\circ_{1.20}$
9-28
220Å
125Å
(c)
(d)
144Å
(e)
(f)
154Å
(g)
(h)

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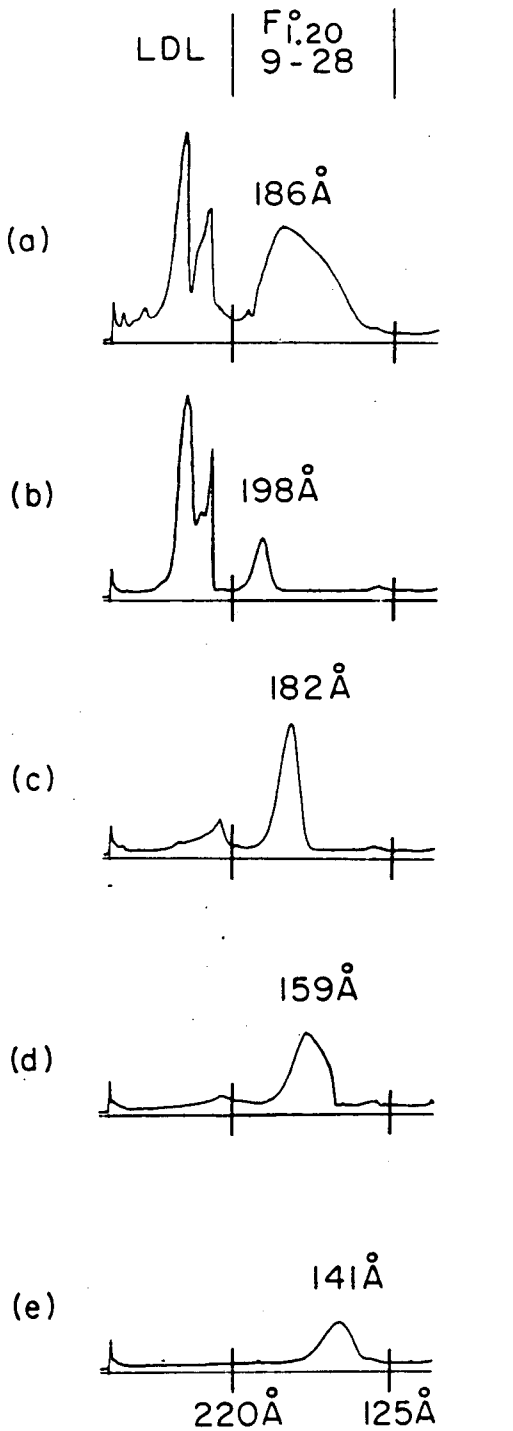
hydrated density range of $F^{o}_{1.20}$9-28 lipoproteins extends up to 1.080 g/ml, the material was isolated at d 1.063 g/ml to reduce the possibility of HDL contamination. Hence, the $F^{o}_{1.20}$9-28 lipoprotein material within the d 1.063-1.080 g/ml fraction was not examined. Figure 9 shows the gradient gel electrophoretic pattern of lipoproteins in the initial serum d 1.006-1.063 g/ml fraction and selected density subfractions from one progeny. The mean particle size of $F^{o}_{1.20}$9-28 material in the d 1.006-1.063 g/ml fraction was 186A. Equilibrium density gradient ultracentrifugation of this fraction yielded seven subfractions with mean densities 1.025, 1.028, 1.034, 1.040, 1.047, 1.056 and 1.066 g/ml. Most of the LDL was distributed in subfractions within the density range of d 1.025 g/ml (top 1 ml) to d 1.034 g/ml (third ml). Material in the particle size range of the $F^{o}_{1.20}$9-28 lipoproteins was found in six subfractions within the density range of d 1.028 g/ml (second ml) to d 1.066 g/ml (bottom ml). Mean particle sizes of the $F^{o}_{1.20}$9-28 lipoproteins in these fractions decreased regularly with increasing density (from 198A at 1.028 g/ml to 141A at 1.066 g/ml). Gradient gel electrophoretic patterns of these lipoproteins in the density range of d 1.028 to 1.040 g/ml exhibited single symmetrical peaks (Figure 9b, c). gradient gel electrophoresis profiles of lipoproteins in fractions with densities ranging from d 1.047 to 1.066 g/ml were not symmetrical (Figure 9d, e), suggesting polydispersity among the $F^{o}_{1.20}$9-28 lipoproteins of higher density. The

Figure 9.

(a). Gradient gel electrophoretic pattern (2-16% gradient gel) of the serum d 1.006-1.063 g/ml fraction, from a progeny with $F^{o}_{1.20}$9-28 lipoprotein concentration of 171 mg/dl, showing both $F^{o}_{1.20}$9-28 lipoprotein material (125-220A) and LDL and IDL material (220-290A).

(b), (c), (d), (e). Electrophoretic patterns of selected density subfractions of the d 1.006-1.063 g/ml fraction in (a) above after equilibrium density gradient ultracentrifugation: (b) d 1.028 g/ml; (c) d 1.040 g/ml; (d) d 1.047 g/ml; and (e) d 1.066 g/ml.



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$F^{o}_{1.20}9\text{-}28$ lipoproteins isolated by density gradient ultracentrifugation from the serum of five progeny exhibited a relationship of decreasing hydrated density similar to that illustrated in Figure 9.

3.4. Apolipoprotein Composition of $F^{o}_{1.20}9\text{-}28$ Lipoproteins

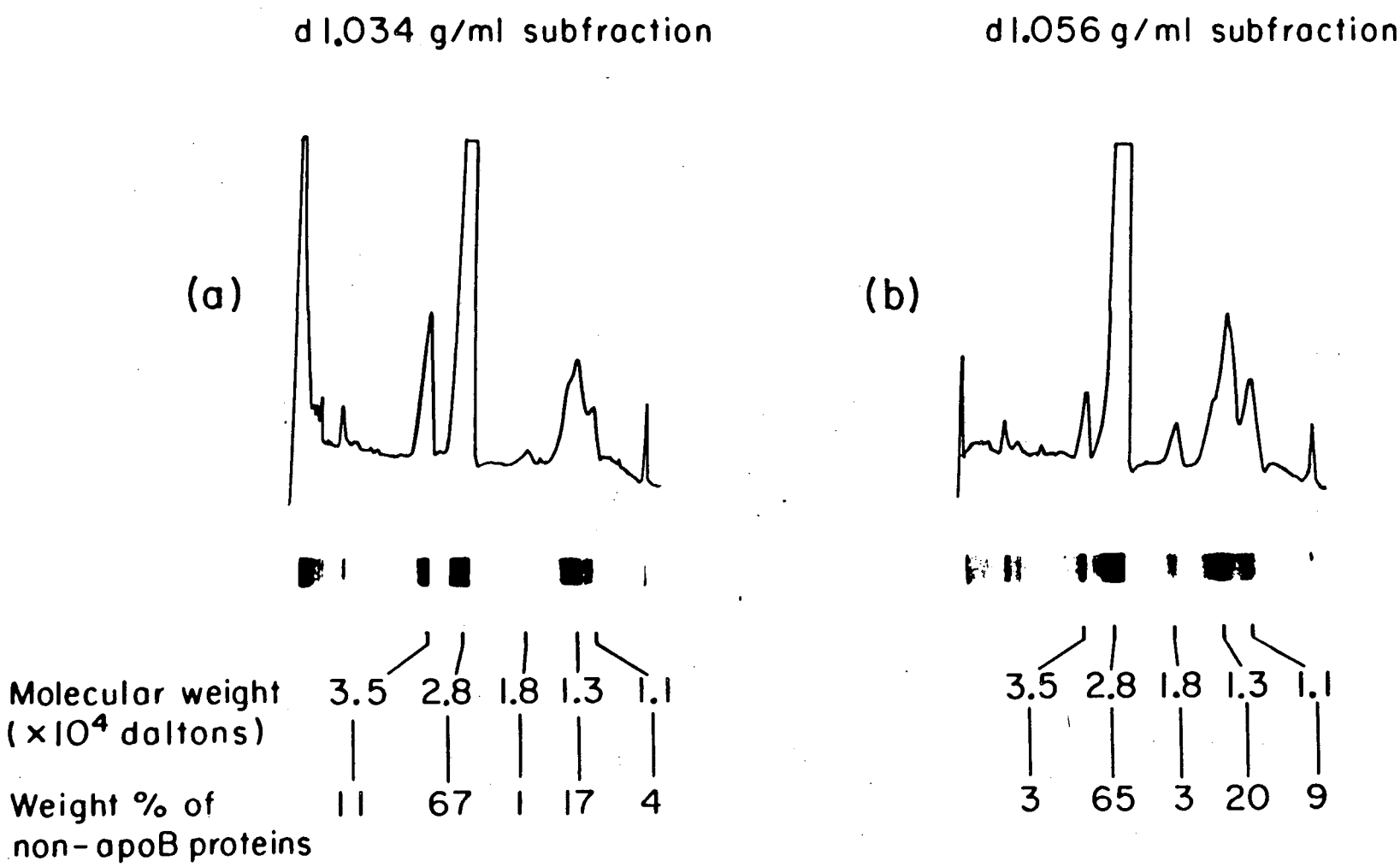
The apolipoprotein composition of each density gradient fraction was analyzed by sodium dodecyl sulfate-polyacrilamide electrophoresis. All fractions, which by gradient gel electrophoresis exhibited $F^{o}_{1.20}9\text{-}28$ material, were found to contain bands at positions corresponding to apoA-I (2.8×10^4 daltons) and apoE (3.5×10^4 daltons). Our identification of these apolipoproteins was supported by radioimmunoassay using antibodies to human apolipoproteins, which also indicated the presence of apoA-I and apoE in all these fractions. Unidentified small molecular weight proteins (approximately 1.8×10^4, 1.3×10^4, and 1.1×10^4 daltons) were also observed by sodium dodecyl sulfate-polyacrilamide electrophoresis. Apolipoprotein B (apoB), observed in the $25\text{-}45 \times 10^4$ dalton range, was detected only in those density fractions exhibiting LDL by gradient gel electrophoresis. In the absence of $F^{o}_{1.20}9\text{-}28$ lipoproteins, the apolipoprotein component of LDL consisted almost exclusively of apoB (apparent apoB: apoE mass ratio always greater than 10:1).

In all, the apolipoprotein content of six density subfractions (mean densities d 1.028-1.066 g/ml) of $F^{o}_{1.20}9\text{-}28$

lipoproteins from the serum of five progeny was examined by sodium dodecyl sulfate-polyacrilamide electrophoresis. The major difference between subfractions was that the less dense subfractions contained relatively more apoE. This trend is illustrated in Figure 10 which shows the sodium dodecyl sulfate-polyacrilamide gels after electrophoresis of apolipoproteins in subfractions with mean densities of 1.034 g/ml (containing $F^{o}_{1.20}9\text{-}28$ lipoproteins with mean particle size of 190A by gradient gel electrophoresis) and 1.056 g/ml (containing $F^{o}_{1.20}9\text{-}28$ lipoproteins with mean particle size of 146A by gradient gel electrophoresis), and the corresponding densitometric scans of these gels. Since the d 1.034 g/ml subfraction also contained some LDL, apoB is apparent at the top of the gel (apparent apoB:apoE mass ratio of 4:1) (Figure 10a). The d 1.040 g/ml subfraction (not shown) contained less LDL, and demonstrated an apparent apoB:apoE mass ratio of 2:1. The d 1.056 g/ml subfraction was essentially free of LDL by gradient gel electrophoresis and showed only a trace of apoB by sodium dodecyl sulfate-polyacrilamide electrophoresis (apparent apoB:apoE mass ratio < 1:1) (Figure 10b). In each subfraction apoA-I constituted approximately 66% of the non-apoB protein. ApoE differed by a factor of 4 between the two subfractions shown in Figure 10 (approximately 11% of total non-apoB protein in the d 1.034 g/ml subfraction vs 3% in the d 1.056 g/ml subfraction). Since some dissociation of apoE from $F^{o}_{1.20}9\text{-}28$ lipoproteins during ultracentrifugation is possible, the

Figure 10.

Sodium dodecyl sulfate-polyacrilamide electrophoretic gels with corresponding densitometric tracings (protein stain) in ultracentrifugal density subfractions (d 1.034 g/ml in (a) and 1.056 g/ml in (b)) from a baboon with $F^{o}_{1.20}9\text{-}28$ concentration of 245 mg/dl. The d 1.034 g/ml subfraction in (a) included some LDL, and apoB was observed near the top of the gel. The major apolipoprotein bands and their percent of non-apoB total protein mass from the densitometric tracings are noted.


d 1.034 g/ml subfraction
d 1.056 g/ml subfraction
(a)
(b)
Molecular weight
(×10⁴ daltons)
3.5 2.8 1.8 1.3 1.1
Weight % of
non-apoB proteins
11 67 1 17 4
3.5 2.8 1.8 1.3 1.1
3 65 3 20 9

above data for apoE content of $F^{o}_{1.20}9\text{-}28$ lipoproteins should be considered as minimum estimates.

3.5. Chemical Composition of $F^{o}_{1.20}9\text{-}28$ Lipoproteins

The $F^{o}_{1.20}9\text{-}28$ lipoproteins in the d 1.006-1.063 g/ml fraction from two progeny were separately fractionated by equilibrium density gradient ultracentrifugation. From the six density subfractions (d 1.028, 1.034, 1.040, 1.047, 1.056, 1.066 g/ml) which contained $F^{o}_{1.20}9\text{-}28$ lipoproteins by gradient gel electrophoresis, the two subfractions of lowest density (d 1.028 and 1.034 g/ml) were not used because they contained considerable amounts of LDL. The three most dense subfractions (d 1.047, 1.056, and 1.066 g/ml) were pooled (to make one subfraction with average hydrated density of 1.056 g/ml) to provide sufficient lipoprotein mass for analysis. The subfraction of density 1.040 g/ml was also analyzed. These two subfractions contained most (>80%) of the $F^{o}_{1.20}9\text{-}28$ lipoprotein material in the d 1.006-1.063 g/ml fraction and contained little or no LDL by gradient gel electrophoresis or apoB by sodium dodecyl sulfate-polyacrilamide electrophoresis. The chemical composition of $F^{o}_{1.20}9\text{-}28$ lipoproteins in the two density gradient subfractions (mean density of 1.040 g/ml, containing $F^{o}_{1.20}9\text{-}28$ lipoproteins with mean particle size of 182A by gradient gel electrophoresis; and 1.056 g/ml with mean particle size of 146A by gradient gel electrophoresis) is shown in Table 8. The major lipid moieties were cholesteryl

Table 8. Chemical composition of two density subfractions of $F^{o}_{1.20}9\text{-}28$ lipoproteins isolated from the sera of two progeny with $F^{o}_{1.20}9\text{-}28$ lipoproteins

Density of Subfraction	Baboon*	Protein	Unesterified Cholesterol	Cholesteryl Ester	Phospholipid	Triglyceride
(g/ml)		(Weight %)				
1.040	A	24.4	7.3	35.1	32.3	0.9
	B	23.9	7.5	33.1	34.7	0.8
1.056	A	30.6	7.1	31.5	30.8	0.0
	B	29.9	6.4	28.9	34.8	0.0

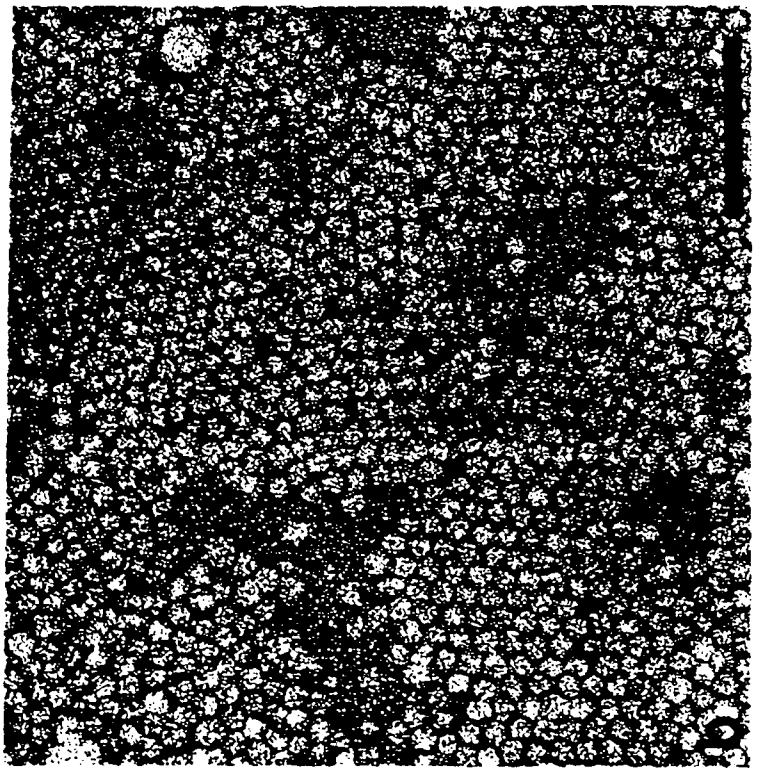
* $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration in serum of Baboon A was 171 mg/dl; concentration in the serum of Baboon B was 207 mg/dl.

ester and phospholipids, each constituting between 29 and 35% of the total lipoprotein mass measured in each subfraction. The protein moiety constituted 24% of the material in the d 1.040 g/ml subfraction and 30% of the d 1.056 g/ml subfraction. For each of the two density subfractions analyzed, there was no difference in chemical composition between the two progeny. Since $F^{o}_{1.20}9\text{-}28$ lipoproteins of all animals (which possessed this lipoprotein class) showed similar size and flotation characteristics, the results in Table 8 should be representative of $F^{o}_{1.20}9\text{-}28$ lipoproteins in the experimental group.

Electron microscopy of the above density fractions from baboon "A" (Figure 11) showed predominantly spherical particles packed in hexagonal array. Mean particle diameters determined by electron microscopy (181A, d 1.040 g/ml subfraction in Figure 11; 138A, d 1.056 g/ml subfraction in Figure 11b) corresponded well with particle diameters obtained by gradient gel electrophoresis (182A, d 1.040 g/ml and 146A, d 1.056 g/ml).

Figure 11.

Electron micrographs of negatively stained $F^{o}_{1.20}{}^{9-28}$ lipoproteins within (a) d 1.040 g/ml subfraction and (b) d 1.056 g/ml subfraction obtained from baboon serum following density gradient ultracentrifugation. Magnification is 240,000x. Bar markers represent 1000A.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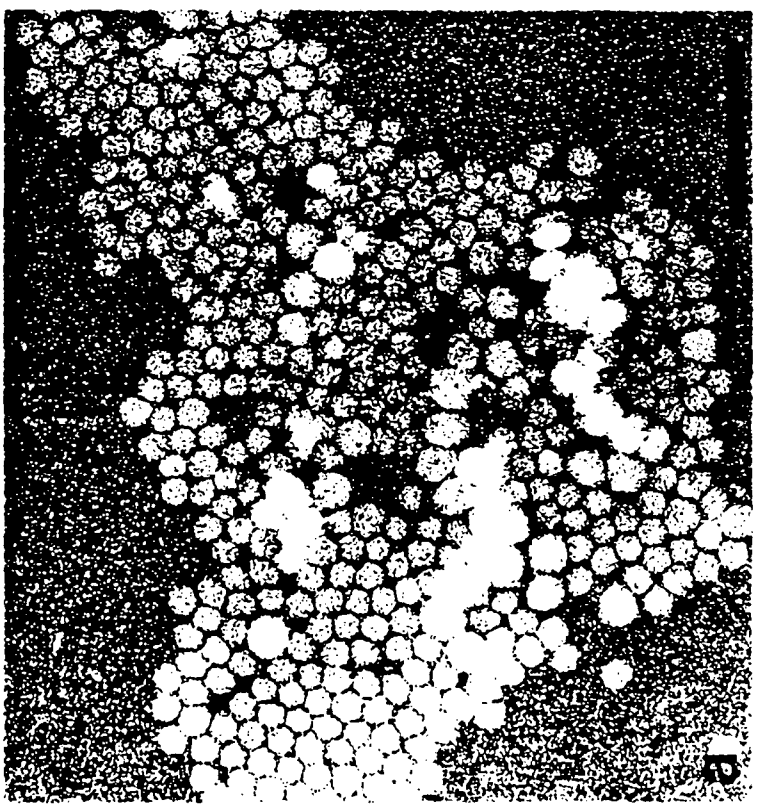
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4. Low Density Lipoproteins (LDL), Intermediate Density Lipoproteins (IDL) and Very Low Density Lipoproteins (VLDL)

4.1. Concentrations of Lipoproteins in the Serum d<1.063 g/ml Fraction

Figure 12 shows the analytic ultracentrifugation schlieren patterns of the 30 minute frame of the serum d≤1.063 g/ml fraction from three progeny in the study. All progeny examined exhibited a single, major peak within the $S^{o}_{f}5$-12 flotation interval, which corresponds well with the flotation rates of LDL in other species (71,86). The LDL, measured as material within the $S^{o}_{f}5$-12 flotation interval, always appears as a single, narrow peak of flotation rate between $S^{o}_{f}7.37$ and $S^{o}_{f}9.56$. Other lipoprotein material sometimes appeared as a single broad peak within the $S^{o}_{f}0$-5 flotation rate interval. This material corresponded to the $F^{o}_{1.20}9$-28 lipoprotein species characterized in the previous section of this dissertation. Small amounts of lipoprotein material were also observed within the $S^{o}_{f}12$-100 flotation interval, which were measured as IDL ($S^{o}_{f}12$-20) and VLDL ($S^{o}_{f}20$-100) material. Only rarely did an animal possess lipoprotein material having flotation rates larger than $S^{o}_{f}100$.

The mean ± SD LDL ($S^{o}_{f}5$-12) concentration of all progeny in this study was 225 ± 95 mg/dl (Table 9); the range of LDL concentrations observed was 88-496 mg/dl among this group of pedigreed progeny consuming the LARD+CS diet. The

Figure 12.

Analytic ultracentrifugal schlieren patterns (a,b,c) and the corresponding gradient gel electrophoretic patterns (2-16% gels) (2-16% gradient gels) of the serum d≤1.063 g/ml fraction of three baboon progeny representing the variety of schlieren patterns of LDL, IDL, and VLDL and the corresponding particle size profiles observed in this study. Additional information on the lipoproteins of these three animals is provided in the text.

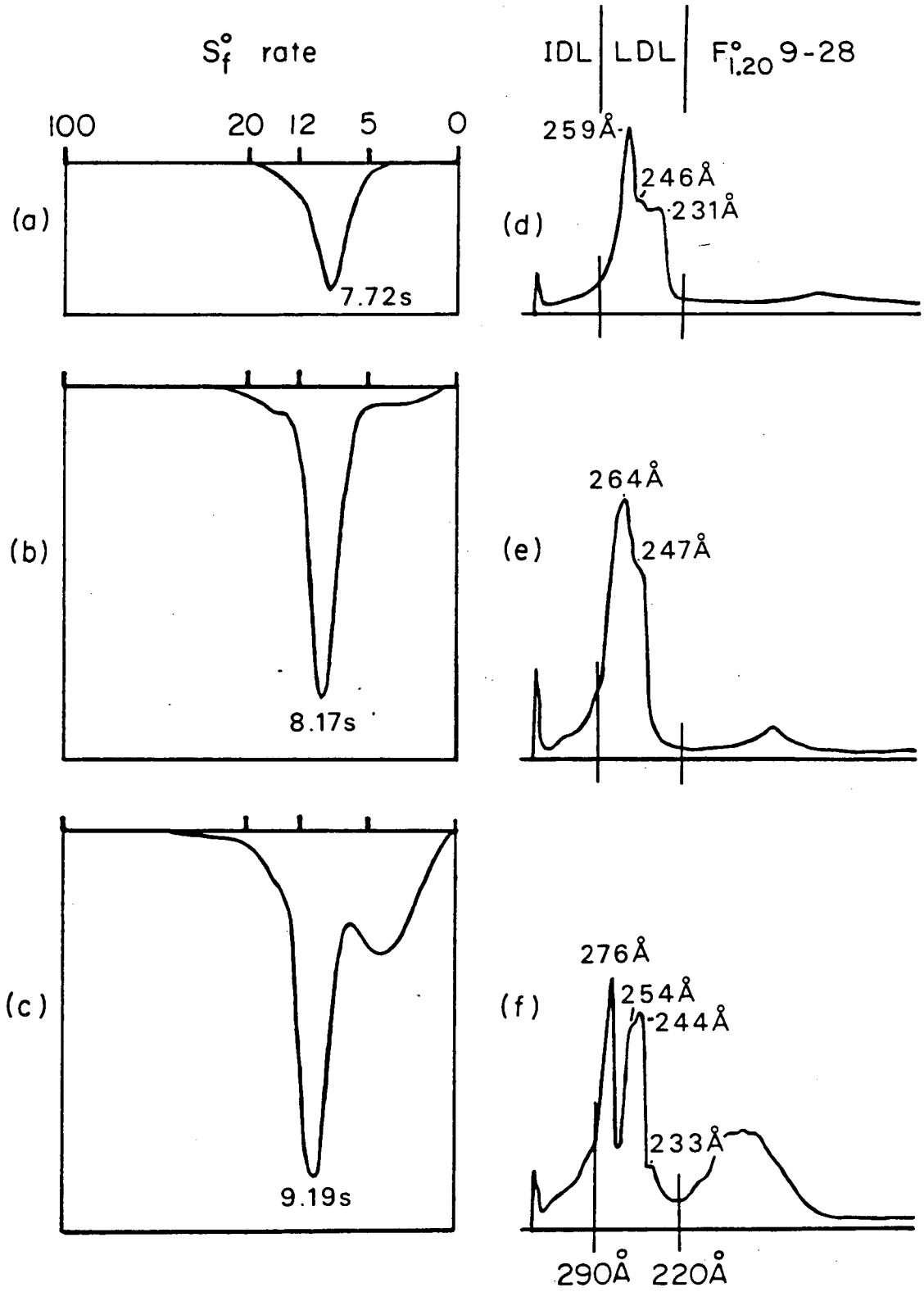

S_f° rate
100
20
12
5
0
(a)
7.72s
(b)
8.17s
(c)
9.19s
IDL
LDL
$F_{1.20}^\circ$ 9-28
259Å
246Å
231Å
(d)
264Å
247Å
(e)
276Å
254Å
244Å
233Å
(f)
290Å
220Å

Table 9. Mean ± SD serum concentrations (mg/dl) of LDL, IDL and VLDL and peak S^{o}_{f} rate (svedbergs) (within the d≤1.063 g/ml fraction) from all pedigreed progeny studied in Experiment I (n=46).

Lipoprotein Species	Group Mean ± SD	Range of Values
LDL (S^{o}_{f}5-12)	225 ± 95	88 - 496
IDL (S^{o}_{f}12-20)	43 ± 38	4 - 170
VLDL (S^{o}_{f}20-100)	9 ± 12	0 - 40
Peak S^{o}_{f} Rate	8.51 ± .56	7.37 - 9.57

peak flotation rate was $S^{o}_{f}8.51 \pm 0.56$ (mean$\pm$SD) with an overall range of values of $S^{o}_{f}7.37$ - $S^{o}_{f}9.57$. IDL ($S^{o}_{f}12$-20) concentrations were quite variable (range of observed values of 4-170 mg/dl with a mean $\pm$ SD of 43 $\pm$ 38 mg/dl) while VLDL levels were quite low (mean $\pm$ SD of 9 $\pm$ 12 mg/dl with an overall range of values of 0-40 mg/dl).

4.2. Statistical Correlations among Lipoproteins within the Serum d<1.063 g/ml Fraction

Possible relationships between lipoproteins within the serum d$\leq$1.063 g/ml fraction were examined by calculating statistical correlation coefficients among concentrations of LDL, IDL, VLDL, and LDL peak flotation (S^{o}_{f}) rate (Table 10). All of these parameters were found to be positively correlated. The manner in which these relationships are reflected in analytic ultracentrifugation schlieren patterns is exhibited in Figure 12. For these representative animals shown in the figure, as LDL ($S^{o}_{f}5$-12) concentrations increased (137 mg/dl in 12a; 292 g/ml in 12b; 392 g/ml in 12c), so did peak S^{o}_{f} rate ($S^{o}_{f}7.72$, 12a; $S^{o}_{f}8.17$, 12b; $S^{o}_{f}9.19$, 12c), as well as IDL (19 mg/dl; 35 mg/dl; 72 mg/dl) and VLDL (1mg/dl; 3mg/dl; 9 mg/dl) concentrations.

4.3. Characterization of LDL Size Distributions by Gradient Gel Electrophoresis

The particle size distribution of lipoprotein material in the serum d$\leq$1.063 g/ml fraction of some progeny in the

Table 10. Coorelation coefficients among lipoprotein species within the serum d≤1.063 g/ml fraction from all pedigreed progeny studied in Experiment I (n=46).

	IDL	VLDL	Peak S^{o}_{f} Rate
LDL	.78[a]	.38[b]	.43[a]
IDL	-	.58[a]	.67[a]
VLDL		-	.64[a]

a: P<0.005
b: P<0.01

study was examined by gradient gel electrophoresis. LDL and $F^{o}_{1.20}9\text{-}28$ lipoprotein material isolated at d 1.063 g/ml are the most prominant species in the gradient gel electrophoresis patterns. As shown in Figure 12(d,e,f) the LDL of progeny in this study appeared in gradient gel electrophoresis patterns as two or more peaks within the 220-290A particle size range. Material of smaller particle size, when observed, was $F^{o}_{1.20}9\text{-}28$ lipoproteins, as described in an earlier section. Material of larger (>290A) particle size generally appeared as small shoulders on the major LDL peaks (see Figure 12f) and probably represented IDL and VLDL material.

Since baboons often exhibit, by gradient gel electrophoresis, several major peaks within the LDL size range it was not possible to make a statistical comparison between the LDL peak flotation rate (by analytic ultracentrifugation) and the LDL peak particle size (by gradient gel electrophoresis). However, there was a general trend, within the population of baboons studied by both methods, of the appearance of major peaks corresponding to material of larger particle size (within the LDL spectrum) for animals which possessed faster LDL peak flotation rates. This trend toward increasing LDL particle size with increasing LDL peak flotation rate is also illustrated in Figure 12. The analytic ultracentrifugation pattern of the first animal (Figure 12a) exhibits a peak flotation rate of $S^{o}_{f}7.72$ while the gradient gel electrophoretic pattern (Figure 12d) shows a

peak particle size of 259A; in Figures 12b,e $S^{o}_{f}8.17$ and 264A; and in Figures 12c,f $S^{o}_{f}9.19$ and 276A.

4.4. Heterogeneity of Lipoproteins within the d 1.006-1.063 g/ml Fraction

In order to characterize the properties of the lipoprotein classes found within the serum d 1.006-1.063 serum fraction, this fraction was isolated from serum of five progeny in the study and was subjected to equilibrium density gradient ultracentrifugation within a final density range of d 1.025 g/ml (top 0.5 ml) to d 1.066 g/ml (bottom 0.5 ml).

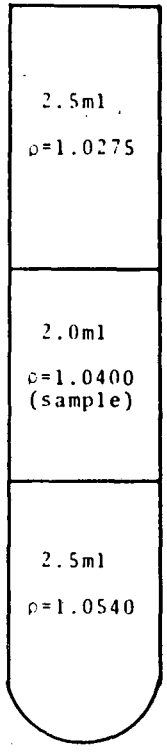
Figure 13 is a photograph of a centrifuge tube following ultracentrifugation and showing the material distribution within the density gradient. All progeny examined demonstrated three distinct lipoprotein bands by this method. First, all progeny exhibited a band of material in the top 0.5 ml of the gradient (Region I: $d \leq 1.025$ g/ml); a second band was observed in the region of the second ml (Region II: d 1.028-1.040 g/ml). Differing amounts of material were also observed spread throughout the lower half of the tube (4th through 7th ml; Region III d 1.040-1.066 g/ml). These visual observations were confirmed when the distribution of protein within the density gradient was measured (Figure 14). Namely, the protein distribution showed a peak at the top 0.5 ml, a second peak near the second ml and a broad distribution in the fractions of greater density.

Figure 13.

Diagram of one of the density gradient ultracentrifugal procedures employed to separate and subfractionate LDL and $F^{o}_{1.20}9\text{-}28$ lipoproteins within the d 1.006-1.063 g/ml serum fraction. The photograph shows the typical banding pattern observed by this procedure: a band at the top of the tube (Region I, $d \leq 1.025$ g/ml), a second band approximately 2 ml from the top of the tube (Region II, d 1.028-1.034), and a diffuse distribution of material in the lower half of the tube (Region III, d 1.034-1.064 g/ml for the baboon shown).

Subfractionation of d 1.006-1.063 g/ml Fraction from Baboon Serum High in $F^{\circ}_{1.20}$ 9-28

Density layers
(pre-centrifugation)



Banding pattern
(post-centrifugation)

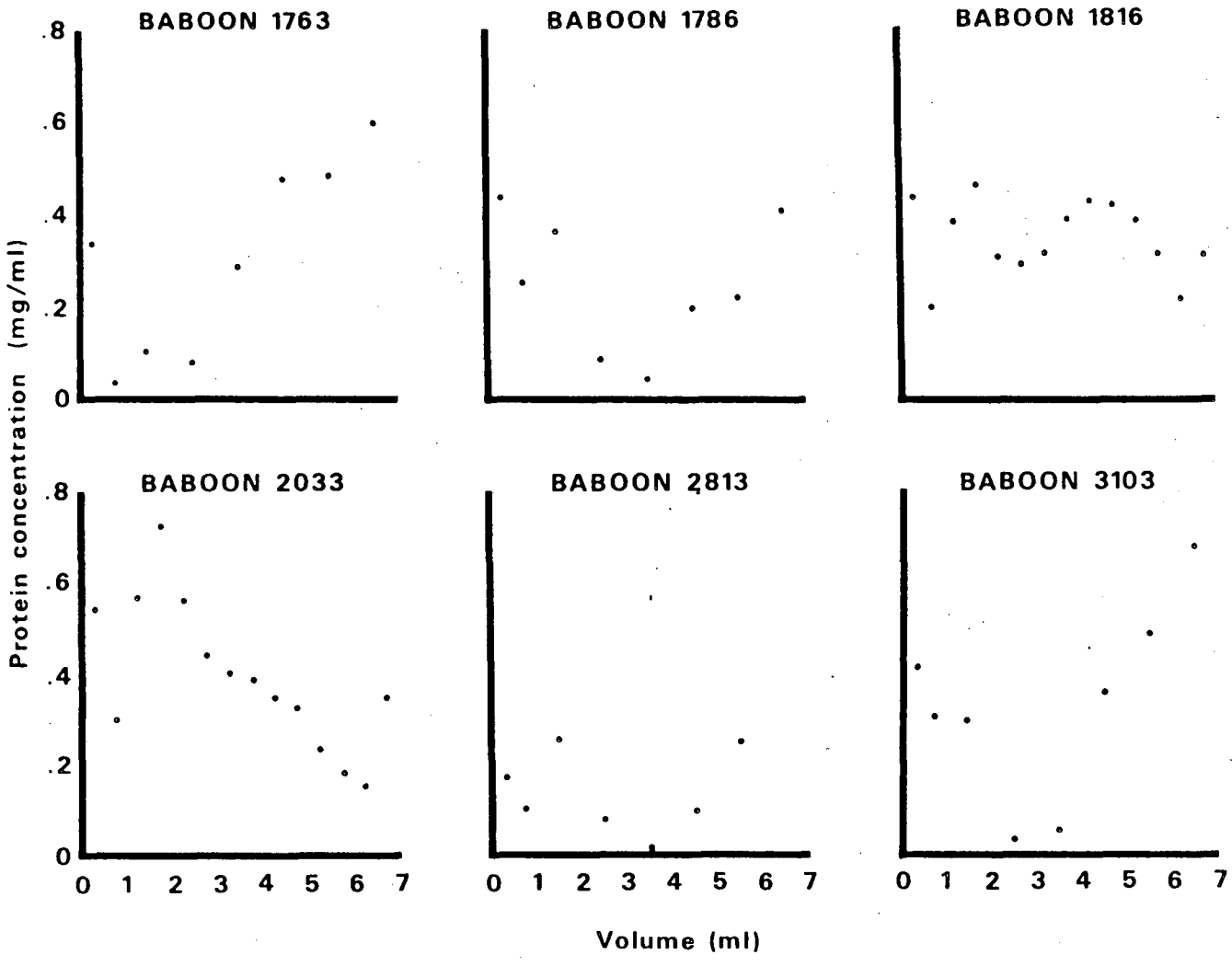
Density gradient
(post-centrifugation)

t	
0-0.5	1.0246
0.5-1.0	1.0261
1.0-1.5	1.0275
1.5-2.0	1.0297
2.0-2.5	1.0319
2.5-3.0	1.0341
3.0-3.5	1.0371
3.5-4.0	1.0400
4.0-4.5	1.0433
4.5-5.0	1.0471
5.0-5.5	1.0527
5.5-6.0	1.0569
6.0-6.5	1.0624
6.5-7.0	1.0644

SW 45 rotor
42 hours, 20°C
174,000 x g

Figure 14

Distribution of protein within the d 1.006-1.063 g/ml serum fraction after density gradient ultracentrifugation of this fraction by the method outlined in Figure 13. Each of the six baboons studied by this method demonstrated a band of protein within the top 0.5 ml of the tube (designated Region I in the text), another band within the second ml of the tube (designated Region II) and a broad band of material in the lower half of the tube (Region III).


BABOON 1763
BABOON 1786
BABOON 1816
BABOON 2033
BABOON 2813
BABOON 3103
Protein concentration (mg/ml)
.8
.6
.4
.2
0
0 1 2 3 4 5 6 7
Volume (ml)

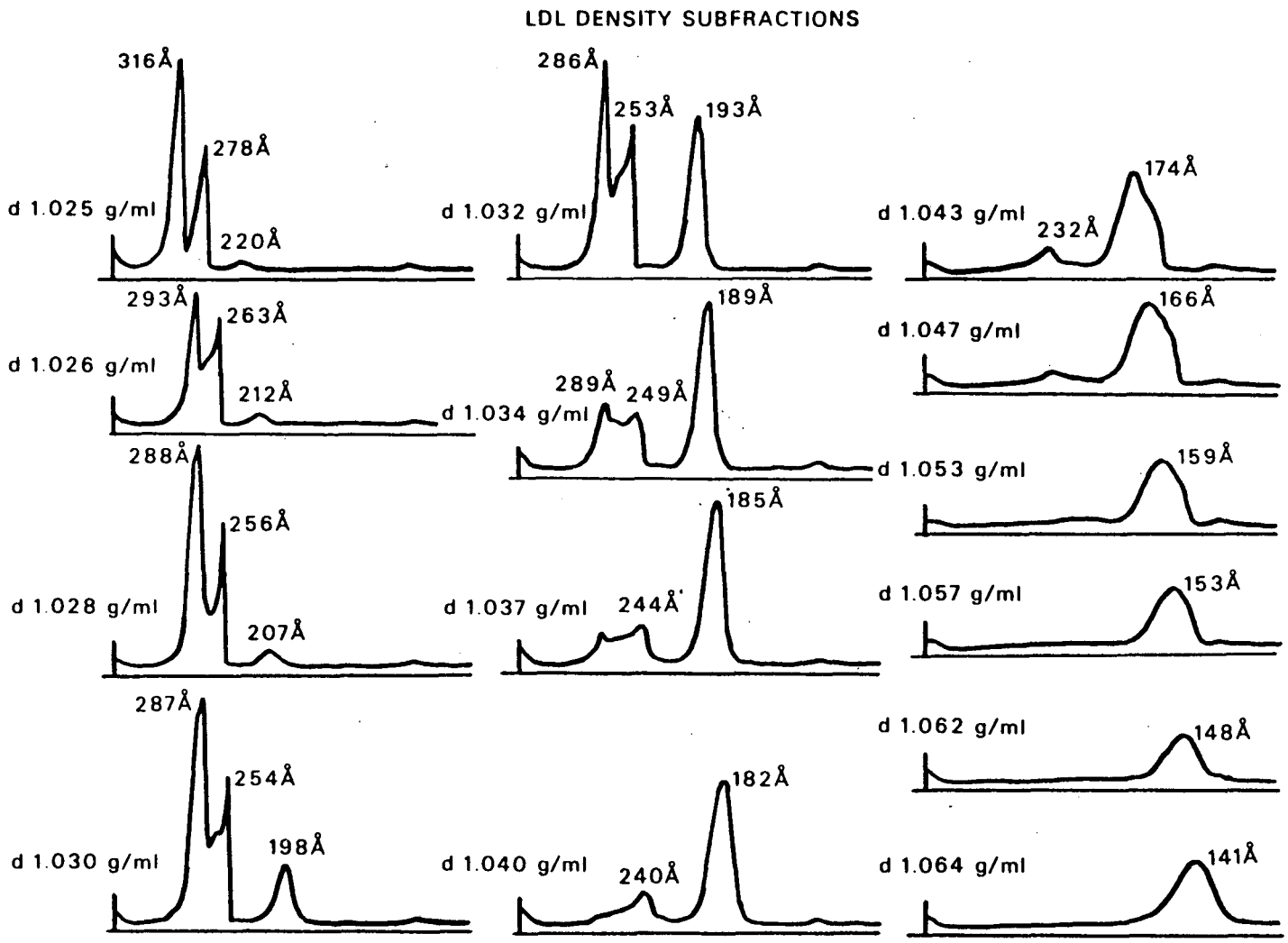
Homogeneity of individual density subfractions was monitored by gradient gel electrophoresis. This also provided preliminary information on the size and heterogeneity of the isolated species. Figure 15 shows gradient gel electrophoresis patterns of density subfractions of the serum d 1.006-1.063 g/ml fraction from one of the five progeny studied. Material in the top three ml (first six density subfractions) was often polydisperse within individual density subfractions. Consequently, density gradient ultracentrifugation did not yield density subfractions within Regions I and II which contained a lipoprotein species of discrete size. There was, however, a general trend of decreasing particle size with increasing hydrated density for subfractions from the serum of each animal. One exception, though, was the appearance of material of particle size ~300A within the d 1.047-1.066 g/ml subfractions of some of the animals. This material was tentatively identified as Lp(a), since species within this density subfraction were observed to cross-react with antibodies raised against human Lp(a). Almost all other material found within Region III was identified as $F^{o}_{1.20}$9-28 lipoproteins by gradient gel electrophoresis.

4.5. Apolipoprotein Composition of Lipoproteins in Regions I, II, III

The apolipoprotein composition of the three lipoprotein bands from all five progeny was examined by sodium dodecyl

Figure 15.

Gradient gel electrophoretic patterns (2-16% gels) of density subfractions of the d 1.006-1.063 g/ml fraction from baboon 2033. Polydisperse LDL (within particle size range 220-290A) is contained within the subfractions of density 1.025-1.037 g/ml, while essentially all the $F^{o}_{1.20}9\text{-}28$ lipoprotein material (within particle size range 125-220A) is found within the subfractions of density 1.028-1.064 g/ml. These patterns illustrate the trend of decreasing particle size with increasing hydrated density apparent for both LDL and $F^{o}_{1.20}9\text{-}28$ lipoproteins. The LDL within individual density subfractions was polydisperse, while $F^{o}_{1.20}9\text{-}28$ lipoproteins were often monodisperse.


LDL DENSITY SUBFRACTIONS
316Å
278Å
220Å
d 1.025 g/ml
286Å
253Å
193Å
d 1.032 g/ml
174Å
d 1.043 g/ml
232Å
293Å
263Å
212Å
d 1.026 g/ml
189Å
289Å
249Å
d 1.034 g/ml
166Å
d 1.047 g/ml
288Å
256Å
207Å
d 1.028 g/ml
185Å
d 1.037 g/ml
244Å
159Å
d 1.053 g/ml
153Å
d 1.057 g/ml
287Å
254Å
198Å
d 1.030 g/ml
182Å
d 1.040 g/ml
240Å
148Å
d 1.062 g/ml
141Å
d 1.064 g/ml

sulfate-polyacrilamide electrophoresis (Figure 16). Lipoproteins within Region I contained almost exclusively apoB, which often appeared as multiple bands within the 25-45 x 10^4 MW size range. Lipoproteins within Region II contained both apoB, similar in appearance to that observed in the lipoproteins of Region I, as well as some smaller (10-20 x 10^4 MW) peptides. Some of the animals also possessed a large amount of an unidentified 6 x 10^4 MW protein in this region. The apolipoproteins observed within Region III were identified as apoA-I and apoE, as well as smaller apolipoproteins, consistent with the presence of $F^{o}_{1.20}9\text{-}28$ lipoproteins within these subfractions. The density subfractions within Region III, which exhibited Lp(a) (as identified by gradient gel electrophoresis and radioimmunoassay) showed, in addition to the apolipoproteins of the $F^{o}_{1.20}9\text{-}28$ lipoproteins present in the subfraction, the presence of apoB near the top of the sodium dodecyl sulfate-polyacrilamide electrophoretic gel. In each case when an animal possessed Lp(a), the apoB contained within Region III appeared to be of higher molecular weight than the apoB appearing within Regions I or II for that animal.

4.6. Chemical Composition of Lipoproteins in Regions I and II

The chemical composition of lipoproteins within Regions I and II was determined for two progeny studied (Table 11). There were minimal differences in composition between the

Figure 16.

Representative sodium dodecyl sulfate-polyacrilamide electrophoretic gels illustrating the apoprotein composition of lipoproteins isolated within density Regions I, II and III. Regions I and II demonstrate apoB as the predominant apolipoprotein moiety, although low amounts of apoE are apparent in each gel. Region II demonstrates the presence of apoB, apoE, apoA-I and other small molecular weight peptides, consistent with the presence of both LDL and $F^{o}_{1.20}{}^{9-28}$ lipoproteins within this density subfraction.

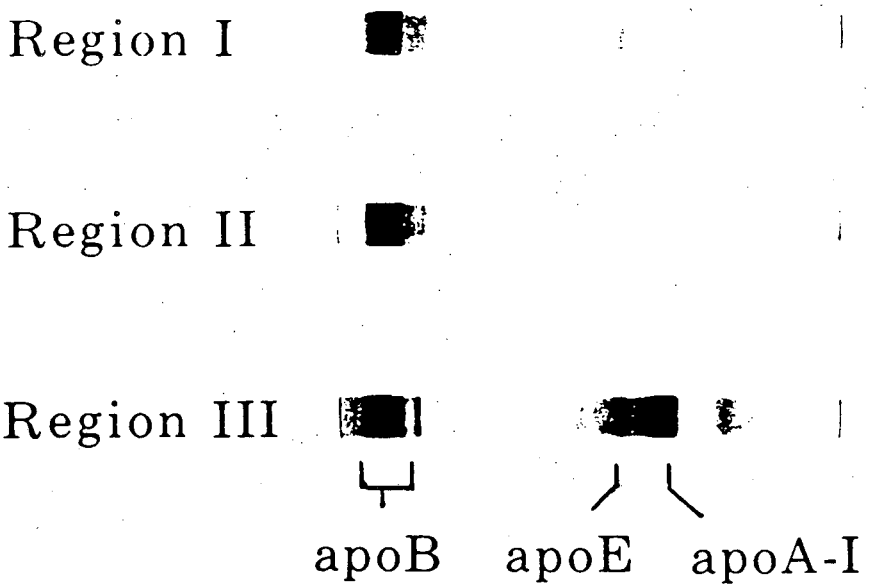

Region I
Region II
Region III
apoB
apoE
apoA-I

Table 11. Chemical composition of the two major density subfractions of baboon LDL (Region I and Region II) isolated from the sera of the same baboons used for Table 8.

Subfraction	Baboon	Protein	Unesterified Cholesterol	Cholesteryl Ester	Phospholipid	Triglyceride
		(Weight %)				
Region I	A	17.2	9.3	45.8	25.5	2.2
	B	17.5	9.3	46.5	24.6	2.1
Region II	A	19.3	9.0	44.2	26.3	1.3
	B	19.7	8.7	44.4	25.8	1.3

material within the two regions. Region I lipoproteins were slightly lower in protein (17.4% vs 19.5% of total lipoprotein mass) and triglyceride (2.2% vs 1.3%) and slightly higher in cholesteryl esters (46.2% vs 44.3%) than were lipoproteins in Region II. Both regions consisted of approximately 9% unesterified cholesterol and 25-26% phospholipid, by weight.

4.7. Statistical Correlations to Lipoproteins within the Serum d<1.20 g/ml Fraction

Relationships between lipoprotein species were examined by calculating correlation coefficients between lipoproteins in the d≤1.063 g/ml serum fraction (LDL, IDL, VLDL, and S^{o}_{f} rate) and those in the d≤1.20 g/ml serum fraction (HDL, HDL-I, HDL-II, HDL-III through HDL-V, and $F^{o}_{1.20}$ rate) and are shown in Table 12. LDL concentrations correlated negatively with total HDL, HDL-I, and HDL-II concentrations, but positively with HDL-III through HDL-V concentrations. IDL concentrations correlated positively with HDL-III through HDL-V concentrations and showed a marginally significant negative correlation with peak $F^{o}_{1.20}$ rate. Peak S^{o}_{f} rate showed only a marginally significant positive correlation with HDL-III through HDL-V concentrations.

Table 12. Correlation coefficients between lipoprotein species within the serum d≤1.063 g/ml fraction and those within the serum d≤1.20 g/ml fraction from all pedigreed progeny studied in Experiment I (n=46).

	LDL	IDL	VLDL	Peak S^{o}_{f} Rate
Total HDL	-.38[a]	-.20	-.16	-.05
HDL-I	-.33[b]	-.22[d]	-.08	-.05
HDL-II	-.37[a]	-.17	-.26[c]	-.10
HDL-III-V	.47[a]	.42[a]	.09	.23[d]
Peak F^{o}_{f} Rate	.05	.00	.23[d]	.08

a: P<0.005

b: P<0.01

c: P<0.05

d: P<0.1

Chapter IV: Effects of Assortative Mating on Lipoprotein Profiles of Baboon Progeny Consuming the LARD+CS Diet

1. Lipoprotein Concentrations and Particle Sizes in Baboon Families

1.1. Concentrations of Total HDL and HDL Subpopulations

Table 13 shows the mean ± SD and median concentrations of lipoproteins within the serum d≤1.20 g/ml fraction from the progeny of each of the five sires in Experiment I. Serum total and non-precipitable ("HDL") cholesterol levels from each of the five sires are listed at the top of each column for reference. There was no significant difference in mean total HDL concentrations between families, even though sire HDL cholesterol concentrations varied considerably. The distribution of lipoprotein material among HDL subpopulations did, however, differ between the progeny of the hypercholesterolemic sires (102, 839 and 1672) and the progeny of the hypocholesterolemic sires (808 and 959). The former group possessed significantly more HDL-I (group mean ± SD of 308 ± 79 mg/dl vs 228 ± 55 mg/dl, $P<0.05$) and significantly less HDL-II (92 ± 22 mg/dl vs 138 ± 34 mg/dl, $P<0.001$), but both groups had similar concentrations of HDL species within the pool of HDL-III through HDL-V (70 ± 14 mg/dl vs 76 ± 18 mg/dl, respectively). These differences in distribution were responsible for differences in mean $F^{o}_{1.20}$ rates of the schlieren peaks of the total HDL for these two

Table 13. Family mean ± SD and median ( ) concentrations (mg/dl) of lipoprotein species within the serum d≤1.20 g/ml fraction and peak $F^{o}_{1.20}$ rate (svedbergs) from all pedigreed progeny studied in Experiment I, grouped by sire.

	Sire				
	959	808	102	839	1672
Number of Progeny	5	5	13	12	11
Total HDL	467 ± 26 (455)	416 ± 59 (409)	485 ± 91 (476)	459 ± 69 (463)	471 ± 93 (481)
HDL-I	261 ± 14 (262)	195 ± 57 (210)	319 ± 85 (297)	296 ± 68 (285)	309 ± 93 (286)
HDL-II	136 ± 28 (125)	140 ± 43 (127)	100 ± 19 (94)	91 ± 25 (91)	85 ± 17 (85)
HDL-III-V	70 ± 12 (66)	81 ± 24 (72)	66 ± 18 (64)	71 ± 14 (68)	74 ± 11 (72)
Peak $F^{o}_{1.20}$ Rate	4.94 ± .26 (4.89)	4.71 ± .64 (4.91)	4.93 ± .13 (4.98)	5.12 ± .24 (5.08)	5.08 ± .21 (5.07)
Sire's Serum Cholesterol (mg/dl)	110	124	183	282	461
Sire's HDL Cholesterol (mg/dl)	48	47	ND	91	120

ND: Not done

groups ($F^{o}_{1.20}5.04 \pm .21$ for the former and $F^{o}_{1.20}4.82 \pm .45$ for the latter, P=0.01).

1.2. Concentrations of $F^{o}_{1.20}9\text{-}28$ Lipoproteins

Mean concentrations of $F^{o}_{1.20}9\text{-}28$ lipoproteins (Table 13) in the serum of the progeny of each of the three hypercholesterolemic sires (102, 839 and 1672) were significantly greater than the concentrations in the serum of the progeny of the two hypocholesterolemic sires (808 and 959). Among the progeny of sire 1672, $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations ranged between 30 and 247 mg/dl; among the progeny of sire 102, between 9 and 289 mg/dl; and among the progeny of sire 839, between 6 and 198 mg/dl. Only three of the 36 progeny of these three hypercholesterolemic sires possessed $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations which were 12 mg/dl or less. In contrast, all ten progeny of the hypocholesterolemic sires had $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations of 12 mg/dl or less.

1.3. Concentrations of Lipoproteins within the Serum d<1.063 g/ml Fraction

Table 14 shows the mean $\pm$ SD and median concentrations for LDL, IDL and VLDL for the progeny of each of the five sires in Experiment I. The mean LDL ($S^{o}_{f}5\text{-}12$) concentration of the progeny of one hypocholesterolemic sire , 959, was significantly lower than the mean level for the progeny of one hypercholesterolemic sire, 1672. Otherwise, the mean

Table 14. Family mean ± SD and median ( ) concentrations (mg/dl) of lipoprotein species within the serum d≤1.063 g/ml fraction and peak S^{o}_{f} rate (svedbergs) from all pedigreed progeny studied in Experiment I, grouped by sire (n=46).

	Sire				
	959	808	102	839	1672
Number of Progeny	5	5	13	12	11
LDL	130 ± 26 (126)	211 ± 110 (166)	208 ± 100 (177)	221 ± 68 (216)	299 ± 86 (293)
IDL	11 ± 7 (9)	42 ± 40 (23)	47 ± 42 (37)	33 ± 29 (18)	63 ± 39 (51)
VLDL	2 ± 2 (2)	10 ± 17 (1)	10 ± 12 (5)	7 ± 13 (1)	14 ± 12 (12)
Peak S^{o}_{f} Rate	8.18 ± .42 (8.24)	8.32 ± .35 (8.37)	8.64 ± .67 (8.74)	8.26 ± .52 (8.16)	8.87 ± .39 (8.85)
Sire's Serum Cholesterol (mg/dl)	110	124	183	282	461
Sire's "LDL" Cholesterol (mg/dl)	62	77	ND	191	341

ND: Not done

LDL concentration for the 36 progeny of the hypercholesterolemic sires was not significantly higher than the mean LDL concentration for the progeny of the two hypocholesterolemic sires (mean ± SD concentrations of 240 ± 93 mg/dl and 171 ± 87 mg/dl for the two groups of progeny, respectively). These measured differences were not statistically significant because one of the five progeny of sire 808 had an LDL concentration of 405 mg/dl and because six of the thirteen progeny of sire 102 had LDL levels below 175 mg/dl. Mean IDL and VLDL concentrations and peak S^{o}_{f} rates showed no significant differences both among families, as well as when the progeny of hypercholesterolemic sires were compared to the progeny of hypocholesterolemic sires.

1.4. Lipoprotein Particle Size Distributions by Gradient Gel Electrophoresis

The particle size distributions of lipoproteins in both the serum $d \leq 1.063$ g/ml and $d \leq 1.20$ g/ml fractions were examined by gradient gel electrophoresis for some of the progeny in this study. Figures 17, 18 and 19 show the gradient gel electrophoretic patterns of the serum $d \leq 1.063$ g/ml fractions electrophoresed in a 2-16% gradient gel from some of the progeny of the three hypercholesterolemic sires (839, 1672 and 102). All progeny of sire 839 (Figure 17) examined (six of the thirteen baboons in this family) exhibited a similar LDL particle size distribution consisting of a major peak of large particle size (268 ± 5A) and one or two minor peaks of

Figure 17.

Gradient gel electrophoretic patterns (2-16% gels) of the serum $d \leq 1.063$ g/ml fractions from six of the twelve progeny of hypercholesterolemic sire 839. All six progeny of this sire exhibited similar LDL particle size distributions consisting of a major peak of large particle size (268 $\pm$ 5A) and one or two minor peaks of smaller particle size (246 $\pm$ 6A). These progeny did exhibit differing levels of $F^{o}_{1.20}9\text{-}28$ lipoproteins (material to the right of the LDL peaks on the patterns).

PROGENY OF SIRE 839

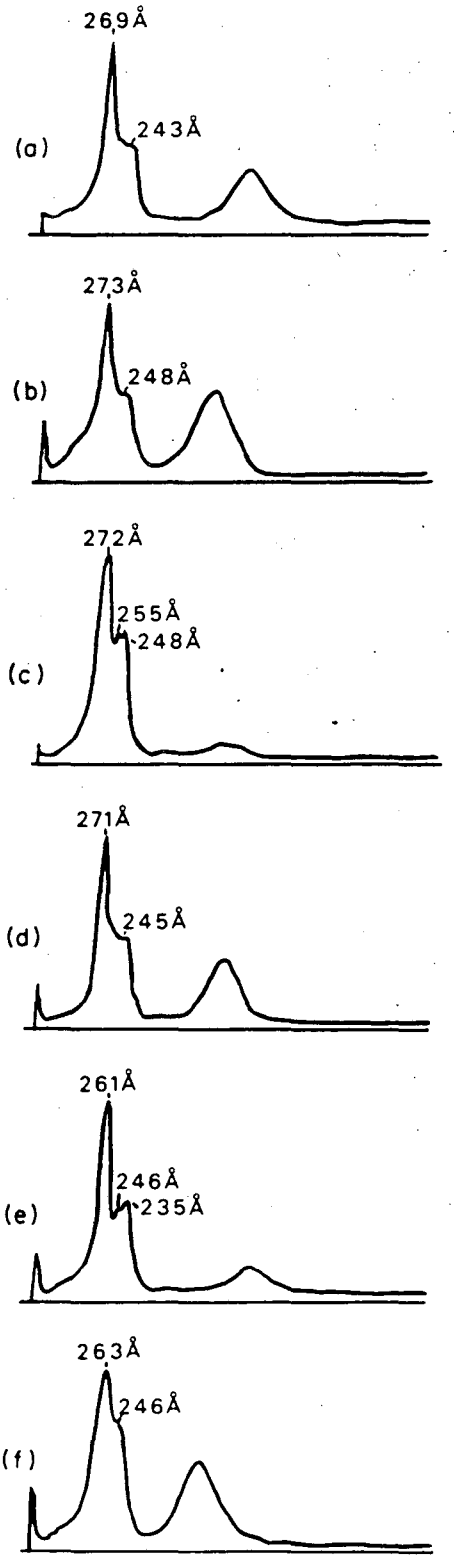


Figure 18.

Gradient gel electrophoretic patterns (2-16% gels) of the serum d≤1.063 g/ml fractions from five of the eleven progeny of hypercholesterolemic sire 1672. These progeny generally exhibited an LDL particle size distribution consisting of a major peak (261 ± 8A) and two minor peaks, one of larger particle size (284 ± 7A) and one of smaller particle size (246 ± 6A). These progeny possessed differing amounts of $F^{o}_{1.20}$9-28 lipoproteins (material to the right of the LDL peaks on the patterns).

PROGENY OF SIRE 1672

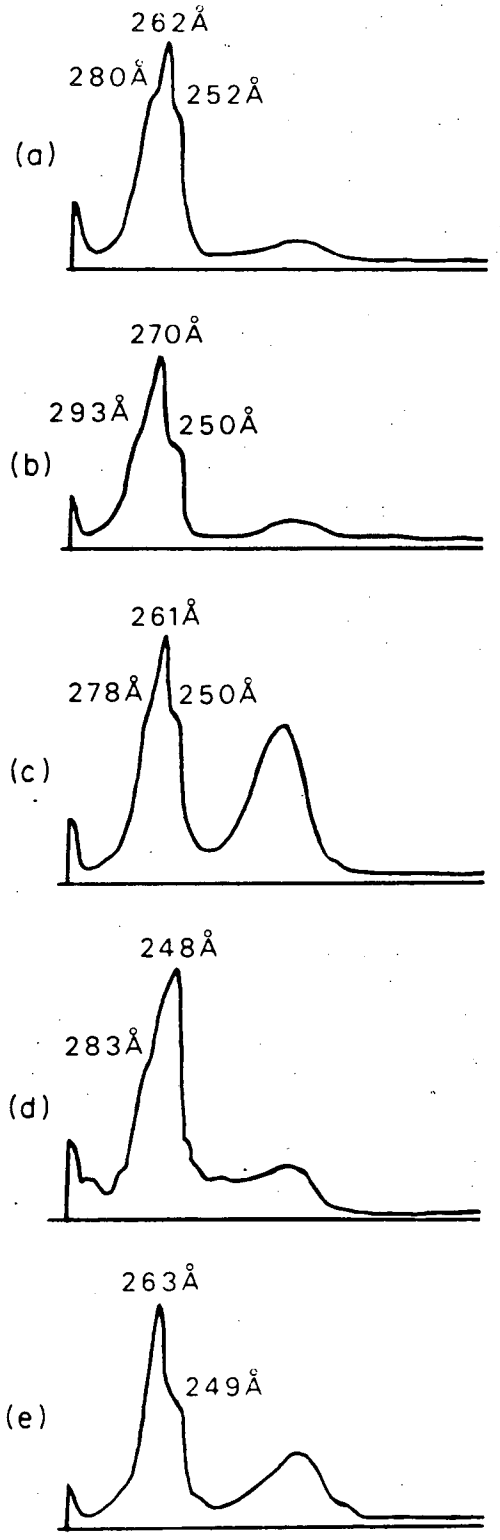
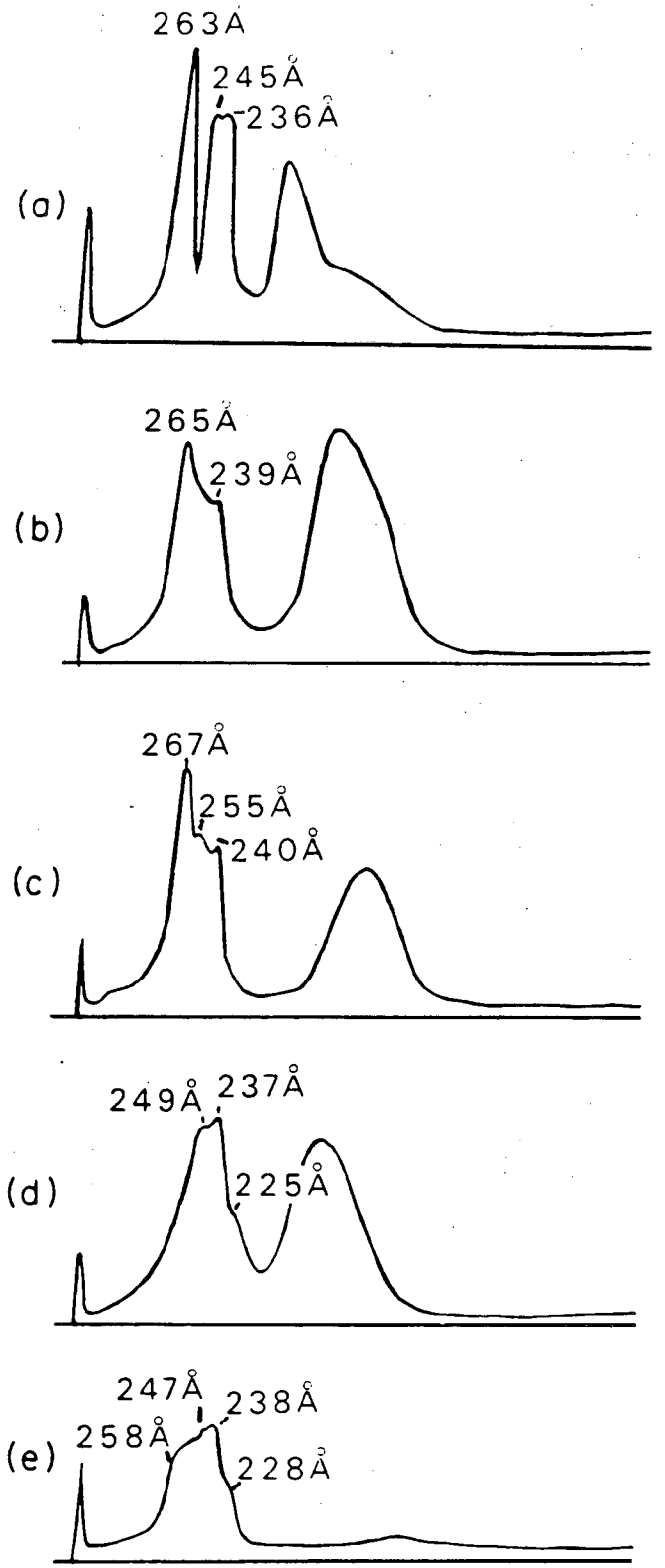


Figure 19.

Gradient gel electrophoretic patterns (2-16% gels) of the serum $d \leq 1.063$ g/ml fractions from five of the thirteen progeny of hypercholesterolemic sire 102. The progeny in (a), (b) and (c) exhibit LDL profiles similar to those of the progeny of sire 839 (Figure 17), consisting of a major peak of particle size 265 $\pm$ 2A and one or two minor peaks of smaller particle size (243 $\pm$ 7A). The progeny in (d) and (e) demonstrate heterogeneous LDL particle size distributions consisting of several peaks, of approximately equal heights, within the 225-258A particle size range.

PROGENY OF SIRE 102



smaller particle size (246 ± 6A). The progeny of sire 1672 (Figure 18) generally exhibited an LDL particle size distribution consisting of a major peak (261 ± 8A) and two minor peaks, one of larger particle size (284 ± 7A) and one of smaller particle size (250 ± 1A) relative to the major peak. The progeny of sire 102 (Figure 19) exhibited two distinct types of patterns. One pattern was similar to the pattern common to the progeny of sire 839: a major peak of particle size 265 ± 2A and one or two minor peaks of smaller particle size (243 ± 7A) (Figure 19a, b and c). The second pattern consisted of a heterogeneous population of lipoprotein material with a particle size range of 225-258A (Figure 19d, e). The progeny of all three hypercholesterolemic sires possessed differing amounts of $F^{o}_{1.20}9\text{-}28$ lipoprotein material, but it always appeared within the 125-220A particle size range.

The particle size distribution of HDL was examined by electrophoresis of the serum $d \leq 1.20$ g/ml fraction obtained from all progeny of all five sires. All progeny exhibited an HDL particle size distribution, similar to that described in Chapter III, Section 1.2, consisting of a major peak of HDL-I and minor peaks corresponding to HDL-II through HDL-V. Examples of this type of particle size distribution can be seen in earlier Figures (Figures 1 and 6).

2. Correlations among Lipoprotein Species in the Serum of Progeny of Hypercholesterolemic Sires

2.1. Correlations among Lipoprotein Species within the Serum d≤1.20 g/ml Fraction

To determine if there are relationships between lipoprotein species within the serum d≤1.20 g/ml peculiar to the progeny of the hypercholesterolemic sires (which were not observed in the data of all progeny (Table 7)) correlation coefficients among lipoprotein levels in this fraction were calculated for all the progeny of the three hypercholesterolemic sires as well as for each of these three families (Table 15). Total HDL concentrations correlated positively with both HDL-I and HDL-II concentrations, but not with HDL-III through HDL-V concentrations. These correlations were observed, to a greater or lesser extent, within each of the three hypercholesterolemic families. HDL-I concentrations were positively correlated to HDL-II concentrations (exclusively due to the strong correlation among the progeny of sire 102) and negatively correlated with HDL-III through HDL-V concentrations. HDL-II concentrations and HDL-III through HDL-V concentrations were positively correlated only among the progeny of sire 1672. HDL-II concentrations were negatively correlated with total HDL peak $F^{o}_{1.20}$ rate among all progeny.

Table 15. Correlation coefficients among the concentrations of lipoprotein species within the serum d≤1.20 g/ml fraction for the progeny of all three hypercholesterolemic sires (n=36) and the progeny of sire 1672 (n=11), sire 839 (n=12) and sire 102 (n=13).

	HDL-I	HDL-II	HDL-III-V	Peak $F^{o}_{1.20}$ Rate
Total HDL				
All progeny	.95[a]	.62[a]	-.08	-.10
Sire 1672	.95[a]	.32	.08	.38
Sire 839	.89[a]	.54[d]	-.32	-.42
Sire 102	.97[a]	.80[a]	-.42	-.06
HDL-I				
All progeny	-	.38[c]	-.32[c]	.01
Sire 1672		.04	-.19	.51
Sire 839		.12	-.65[c]	-.28
Sire 102		.70[b]	-.58[c]	-.08
HDL-II				
All progeny		-	.17	-.46[b]
Sire 1672			.84[b]	-.39
Sire 839			.30	-.46
Sire 102			-.35	-.22
HDL-III-V				
All progeny			-	-.01
Sire 1672				-.49
Sire 839				.14
Sire 102				.35

a: $P<0.005$
b: $P<0.01$
c: $P<0.05$
d: $P<0.1$

2.2. Correlations among Lipoprotein Species within the Serum d<1.063 g/ml Fraction

Table 16 lists the correlation coefficients among LDL, IDL and VLDL concentrations and peak S^{o}_{f} rate for all the progeny of the three hypercholesterolemic sires as well as for each of these three families. Just as was observed for all progeny in the study (Table 10), positive correlations ($P<0.01$ for the group consisting of all 36 progeny) were observed among all these components within the serum d≤1.063 g/ml fraction. Among the progeny of sire 1672, though, LDL concentrations did not correlate with either VLDL concentrations or peak S^{o}_{f} rate.

2.3. Correlations among Lipoprotein Species within the Serum d<1.20 g/ml Fraction and Those within the Serum d<1.063 g/ml Fraction

Table 17 lists the correlation coefficients among lipoprotein species in the serum d≤1.20 g/ml fraction and those within the serum d≤1.063 g/ml fraction for all the progeny of the three hypercholesterolemic sires as well as for each of the three families. LDL concentrations were negatively correlated with total HDL concentrations, being negatively correlated with HDL-I concentrations but positively correlated with HDL-III through HDL-V concentrations (all correlations $P<0.01$ for the group of all 36 progeny). A negative correlation between LDL concentrations and HDL-II concentrations was observed only among the progeny of sire

Table 16. Correlation coefficients among the concentrations of lipoprotein species within the serum d≤1.063 g/ml fraction for the progeny of all three hypercholesterolemic sires (n=36) and the progeny of sire 1672 (n=11), sire 839 (n=12) and sire 102 (n=13).

	IDL	VLDL	Peak S^{o}_{f} Rate
LDL			
All Progeny	.74[a]	.56[a]	.51[b]
Sire 1672	.71[c]	.34	.07
Sire 839	.77[b]	.62[c]	.57[d]
Sire 102	.75[b]	.62[c]	.67[c]
IDL			
All Progeny	-	.57[a]	.68[a]
Sire 1672		.56[d]	.57[d]
Sire 839		.93[a]	.83[a]
Sire 102		.82[a]	.70[b]
VLDL			
All Progeny		-	.55[a]
Sire 1672			.86[a]
Sire 839			.78[a]
Sire 102			.74[b]

a: $P<0.005$
b: $P<0.01$
c: $P<0.05$
d: $P<0.1$

Table 17. Corrrelation coefficients between lipoprotein species within the serum d≤1.063 g/ml fraction and those within the serum d≤1.20 g/ml fraction for the progeny of all three hypercholesterolemic sires (n=36) and the progeny of sire 1672 (n=11), sire 839 (n=12) and sire 102 (n=13).

	LDL	IDL	VLDL	Peak S^o_f Rate
HDL				
All Progeny	$-.42^b$	-.15	-.14	.01
Sire 1672	$-.57^d$	-.14	.26	.18
Sire 839	-.07	.08	.11	-.10
Sire 102	$-.57^c$	$-.74^b$	$-.61^c$	$-.60^c$
HDL-I				
All Progeny	$-.51^b$	$-.32^c$	-.23	-.12
Sire 1672	$-.66^c$	-.29	-.07	-.05
Sire 839	-.33	-.26	-.17	-.23
Sire 102	$-.62^c$	$-.80^b$	$-.68^c$	$-.72^b$
HDL-II				
All Progeny	-.13	.23	.09	.17
Sire 1672	.13	.49	$.73^c$	$.62^c$
Sire 839	.43	$.59^c$	$.52^d$	.22
Sire 102	$-.48^d$	-.38	-.35	-.35
HDL-III-V				
All Progeny	$.50^b$	$.56^a$	$.33^c$	$.51^b$
Sire 1672	.39	$.73^c$	$.74^b$	$.56^d$
Sire 839	.41	$.55^d$	.44	.20
Sire 102	$.56^c$	$.47^d$	$.53^c$	$.72^b$
Peak $F^o_{1.20}$ Rate				
All Progeny	-.23	$-.34^c$	-.11	-.21
Sire 1672	$-.85^a$	$-.78^b$	-.43	-.25
Sire 839	-.31	-.19	-.22	-.05
Sire 102	-.05	.06	.04	-.12

a: P<0.005, b: P<0.01, c: P<0.05, d: P<0.1

102 ($P<0.1$). IDL concentrations were negatively correlated with HDL-I concentrations (mainly among the progeny of sire 102) ($P<0.01$ among the progeny of sire 102, $P<0.05$ among all progeny) and positively correlated with HDL-III through HDL-V concentrations ($P<0.005$ among all progeny). IDL concentrations were also negatively correlated with the HDL peak $F^{o}_{1.20}$ rate ($P<0.05$ among all progeny), exclusively due to the strong correlation ($P<0.01$) observed among the progeny of sire 1672. VLDL concentrations were positively correlated with HDL-III through HDL-V concentrations ($P<0.05$), an observation not seen among all progeny in the study (Table 11). LDL peak S^{o}_{f} rate was also positively correlated ($P<0.01$) with HDL-III through HDL-V concentrations. Other correlations were apparent only among the progeny of individual sires and not among the progeny of all three hypercholesterolemic sires, when taken as a single group.

2.4. Correlations among $F^{o}_{1.20}9\text{-}28$ Lipoproteins and Other Lipoprotein Species

Correlation coefficients between $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations and all other lipoprotein species were calculated for the group consisting of all the progeny of the three hypercholesterolemic sires as well as for each of the three families (Table 18). Within these hypercholesterolemic families, there was an inverse relationship between concentrations of $F^{o}_{1.20}9\text{-}28$ lipoproteins and

Table 18. Correlation coefficients between the concentration of $F^{o}_{1.20}9\text{-}28$ lipoproteins and all other lipoprotein species for the progeny of all three hypercholesterolemic sires (n=36) and the progeny of sire 1672 (n=11), sire 839 (n=12) and sire 102 (n=13).

	All Progeny	Sire 1672	Sire 839	Sire 102
HDL	-.60[a]	-.65[c]	-.69[c]	-,73[b]
HDL-I	-.43[b]	-.20	-.14	-.73[b]
HDL-II	-.54[b]	-.50	-.87[a]	-.77[b]
HDL-III-V	.08	-.27	-.27	.56[c]
Peak $F^{o}_{1.20}$ Rate	-.08	.03	.40	.00
LDL	.16	.21	-.29	.46
IDL	.10	-.05	-.63[c]	.32
VLDL	-.01	-.21	-.56[d]	.29
Peak S^{o}_{f} Rate	.08	-.04	-.40	.67[c]

a: $P<0.005$

b: $P<0.01$

c: $P<0.05$

d: $P<0.1$

concentrations of total HDL ($P<0.05$ in all families), HDL-II ($P<0.01$ in two of the three families) and in HDL-I ($P<0.01$ in one of the three families). These inverse relationships also proved to be statistically significant when the progeny of these three families were treated as a single group ($P<0.01$). Progeny of sire 102 also exhibited a positive correlation between concentrations of $F^{o}_{1.20}$9-28 lipoproteins and peak LDL flotation (S^{o}_{f}) rate ($P<0.05$).

Chapter V: Separate Effects of Saturated Fat and Cholesterol on Lipoprotein Profiles of Pedigreed Baboon Progeny Consuming the LARD+CS Diet

1. High Density Lipoproteins and $F^{o}_{1.20}9\text{-}28$ Lipoproteins

1.1. HDL Concentrations Measured by Analytic Ultracentrifugation

Table 19 lists the mean ± SD concentrations for HDL ($F^{o}_{1.20}0\text{-}9$), HDL-I (approximately $F^{o}_{1.20}4\text{-}9$), HDL-II (approximately $F^{o}_{1.20}2\text{-}4$), and HDL-III through V (approximately $F^{o}_{1.20}0\text{-}2$) for the four progeny of sire 102 fed the sequence of diets previously outlined in Table 3. After consuming the LARD+CS (lard with cholesterol) diet since weaning (Period 1), this group exhibited a mean HDL concentration (412 mg/dl) slightly lower than the mean of all the progeny of sire 102 (see Table 13). When cholesterol was removed from the diet for six weeks (LARD-CS (lard without cholesterol) diet, Period 2), the mean total HDL concentration increased by 34% relative to the mean concentration on the previous diet (LARD+CS diet, Period 1) due to a 62% increase in HDL-I (both $P<0.005$). There was a 29% decrease in mean concentration of HDL-III through HDL-V ($P<0.005$); the mean HDL-II concentration was unchanged. The increase in HDL-I material was accompanied by a 10% increase in the HDL peak flotation rate ($P<0.05$).

Table 19. Mean ± SD values of the concentrations (mg/dl) of total HDL and HDL subpopulations and the peak $F^{o}_{1.20}$ rate (svedbergs) for four progeny of sire 102 during the sequence of dietary periods described in Table 3. All values were determined at the end of each diet period.

Period	Diet	HDL	HDL-I	HDL-II	HDL-III-V	Peak $F^{o}_{1.20}$ Rate
1	LARD+CS*	412 ± 59	249 ± 39	114 ± 16	49 ± 10	4.96 ± .12
2	LARD-CS**	551 ± 48	403 ± 33	113 ± 13	35 ± 7	5.48 ± .31
3	CHOW***	412 ± 63	243 ± 55	129 ± 19	40 ± 10	4.56 ± .42
4	LARD-CS	545 ± 95	383 ± 97	127 ± 13	36 ± 6	5.43 ± .54
5	LARD+CS	507 ± 59	335 ± 66	124 ± 4	48 ± 10	5.05 ± .39

* LARD+CS diet, column 1 of Table 2

** LARD-CS diet, column 2 of Table 2

**** CHOW diet, column 3 of Table 2

When the amount and degree of saturation of the dietary fat was reduced for 12 weeks (CHOW diet, Period 3),the mean total HDL concentration decreased significantly ($P<0.05$) relative to levels on the LARD-CS diet during Period 2. This decrease was due entirely to a 40% decrease in the mean HDL-I concentration. Since HDL-II and HDL-III through HDL-V concentrations increased slightly ($P<0.1$), the HDL peak $F^{o}_{1.20}$ rate decreased by almost 17% ($P<0.005$). Relative to the LARD+CS diet (Period 1) only the mean HDL-III through HDL-V concentration was significantly different (lower) on the CHOW diet ($P<0.005$).

During the six weeks of Period 4, the progeny consumed the LARD-CS diet; the same diet as during Period 2. The mean total HDL concentration during Period 4 was increased ($P<0.01$) relative to the CHOW diet (Period 3), as was the mean HDL-I concentration ($P<0.05$) and the mean HDL peak flotation rate ($P<0.01$). There were no significant changes in the concentrations of the other HDL subpopulations. HDL levels attained during Period 4 (LARD-CS diet following the CHOW diet) were very similar to those observed during Period 2 (LARD-CS diet following the LARD+CS diet) except that the mean HDL-II concentration was higher during Period 4 ($P<0.01$).

In the final six weeks of the study (Period 5, LARD+CS diet) the mean total HDL concentration decreased by only 7% ($P<0.1$), relative to the previous diet, and was 21% greater than the mean level observed during Period 1 on the same

diet ($P<0.01$). Likewise, with the addition of cholesterol to the diet, the mean HDL-I concentration decreased by only 13% ($P<0.1$, LARD+CS diet relative to LARD-CS diet). Consequently, after six week on the LARD+CS diet (Period 5) the mean HDL-I concentration was 35% higher ($P<0.05$) than when these progeny were fed the same LARD+CS diet (for a much longer period of time) during Period 1. HDL-III through HDL-V concentrations, however, did increase considerably ($P<0.005$) during Period 5 (relative to Period 4) and reached the same levels observed during Period 1.

1.2. $F^{o}_{1.20}9\text{-}28$ Lipoprotein Concentrations Measured by Analytic Ultracentrifugation

Table 20 lists the mean $\pm$ SD values of the concentrations of $F^{o}_{1.20}9\text{-}28$ lipoproteins and lipoprotein concentrations within selected subintervals of the $F^{o}_{1.20}9\text{-}28$ interval: $F^{o}_{1.20}9\text{-}14$, $F^{o}_{1.20}14\text{-}20$, $F^{o}_{1.20}20\text{-}28$. The initial (Period 1) mean concentration of $F^{o}_{1.20}9\text{-}28$ lipoproteins within this group of progeny (232 mg/dl) was higher than the mean concentration of these lipoprotein species among all the progeny of sire 102 (163 mg/dl, as shown in Table 13) which is consistent with the previous observations (Table 18 in Chapter III) which showed an inverse relationship between HDL and $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations.

When cholesterol was removed from the diet for six weeks (LARD-CS diet, Period 2), the mean $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration decreased by 58% ($P<0.05$) relative to

Table 20. Mean ± SD concentrations (mg/dl) of $F^{o}_{1.20}9\text{-}28$ lipoproteins and of three selected flotation subintervals for four progeny of sire 102 during the sequence of dietary periods described in Table 3. All values were determined at the end of each diet period.

Period	Diet	$F^{o}_{1.20}9\text{-}28$	$F^{o}_{1.20}9\text{-}14$	$F^{o}_{1.20}14\text{-}20$	$F^{o}_{1.20}20\text{-}28$
1	LARD+CS*	232 ± 63	86 ± 26	104 ± 33	42 ± 20
2	LARD-CS**	98 ± 51	85 ± 42	14 ± 10	0 ± 0
3	CHOW***	12 ± 11	12 ± 11	0 ± 0	0 ± 0
4	LARD-CS	42 ± 33	40 ± 31	2 ± 2	0 ± 0
5	LARD+CS	130 ± 86	84 ± 25	33 ± 31	15 ± 30

* LARD+CS diet, column 1 in Table 2

** LARD-CS diet, column 2 in Table 2

*** CHOW diet, column 3 in Table 2

that on the LARD+CS diet. There was a loss of all material within the $F^{o}_{1.20}$20-28 flotation interval ($P<0.05$) and an 87% decrease in material floating within the $F^{o}_{1.20}$14-20 interval ($P<0.01$). After the progeny consumed the CHOW diet for the next 12 weeks (Period 3), $F^{o}_{1.20}$9-28 lipoproteins were essentially eliminated from the plasma of all progeny (mean $\pm$ SD of 12 $\pm$ 11 mg/dl, with greatest concentration of 26 mg/dl). All $F^{o}_{1.20}$9-28 lipoprotein material present was confined to the $F^{o}_{1.20}$9-14 flotation interval.

When the saturated fat was added back to the diet for six weeks (LARD-CS diet, Period 4), the mean $F^{o}_{1.20}$9-28 lipoprotein concentration increased significantly ($P=0.05$, relative to the CHOW diet) but to only 43% of the mean level which these progeny exhibited on the LARD-CS diet during Period 2 ($P<0.05$).

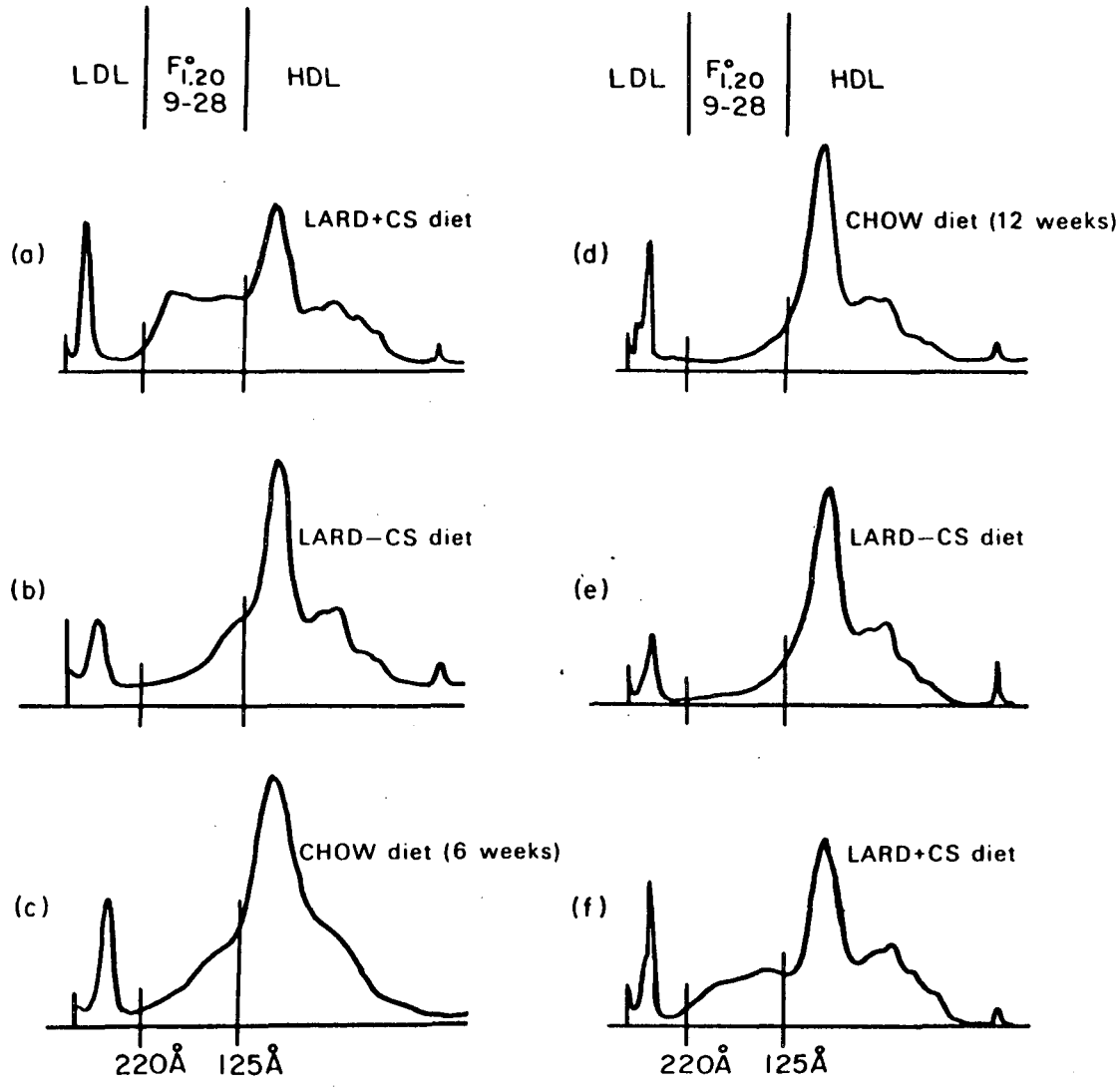
When cholesterol was then added back to the diet (LARD+CS diet, Period 5), the mean $F^{o}_{1.20}$9-28 lipoprotein concentration increased more than three-fold ($P<0.1$) relative to that on the LARD-CS diet (Period 4). After six weeks on the LARD+CS diet, however, the mean $F^{o}_{1.20}$9-28 lipoprotein concentration was still significantly lower ($P<0.05$) than the mean level observed during Period 1, when the progeny were consuming the same LARD+CS diet, but for a longer time period. The main differences were in amounts of material within the faster floating $F^{o}_{1.20}$14-20 ($P<0.01$) and $F^{o}_{1.20}$20-28 ($P<0.1$) subintervals.

1.3. HDL and $F^{o}_{1.20}9\text{-}28$ Lipoprotein Distributions Measured by Gradient Gel Electrophoresis

Figure 20 shows densitometric scans of 4-30% gradient gels of the serum ultracentrifuged d≤1.20 g/ml fraction from one representative progeny (animal 2092) in this study while consuming each diet. On the LARD+CS diet (Period 1, Figure 20a), the HDL appeared as five distinct peaks within the 72-125A particle size range, similar to that of other progeny on this diet (see Figures 1 and 6 for examples). The $F^{o}_{1.20}9\text{-}28$ lipoproteins were observed as a broad distribution of material within the 125-220A particle size range. In some cases this distribution of material demonstrated profiles indicative of subpopulations. After consuming the LARD-CS diet for six weeks (Figure 20b) during Period 2, the major HDL peak (HDL-I) was quite prominant in the electrophoretic pattern. In contrast, the HDL material of smaller particle size did not exhibit discrete species, and material corresponding to the HDL-V subpopulation was not detectable. These observations were consistent with analytic ultracentrifugal results for this dietary period (previous section) which indicated greater HDL-I concentrations and lower HDL-III through HDL-V concentrations on this diet, relative to other diets. In addition, the gradient gel electrophoretic scans for the progeny on the LARD-CS diet indicated lesser amounts of $F^{o}_{1.20}9\text{-}28$ lipoprotein material (material within the 125-220A particle size range) than when on the LARD+CS diet. Most of the $F^{o}_{1.20}9\text{-}28$ lipoprotein material detected

Figure 20.

Gradient gel electrophoretic patterns (4-30% gels) of the serum $d \leq 1.20$ g/ml fraction from one representative baboon (animal 2092) during the sequence of dietary periods outlined in Table 3. In (a), after consuming LARD+CS diet for over a year, the baboon exhibits a considerable amount of $F^{o}_{1.20}9\text{-}28$ lipoprotein material within the 125-220A particle size range. After 6 weeks on the LARD-CS diet (b), the amount of $F^{o}_{1.20}9\text{-}28$ lipoprotein material is reduced. The amount of $F^{o}_{1.20}9\text{-}28$ lipoprotein material changes little after 6 weeks on the CHOW diet (c), but decreases to an almost negligible amount after 12 weeks on the CHOW diet (d). After 6 weeks on the LARD-CS diet (e), the $F^{o}_{1.20}9\text{-}28$ lipoprotein material appears little changed, but increases considerably after 6 weeks on the LARD+CS diet. Additional information is provided in the text..


LDL
$F^{\circ}_{1.20}$ 9-28
HDL
(a)
LARD+CS diet
(b)
LARD−CS diet
(c)
CHOW diet (6 weeks)
220Å
125Å
LDL
$F^{\circ}_{1.20}$ 9-28
HDL
(d)
CHOW diet (12 weeks)
(e)
LARD−CS diet
(f)
LARD+CS diet
220Å
125Å

on the LARD-CS diet was within the smaller particle size ranges (125-160A).

When the progeny on the CHOW diet (for six weeks, halfway through Period 3, Figure 20c) were sampled, the gradient gel electrophoretic patterns of the serum d≤1.20 g/ml fraction were very similar to those for the preceding LARD-CS diet. Namely, HDL-I was the prominant subpopulation, other subpopulations of smaller particle size generally did not appear as discrete species and HDL-V material was essentially absent (Figure 20c). In addition, a peak of $F^{o}_{1.20}9\text{-}28$ lipoprotein material was still observed within the 125-220A particle size range. After 12 weeks on the CHOW diet, however, the gradient gel electrophoretic patterns of the HDL material again showed 4 - 5 distinct peaks and $F^{o}_{1.20}9\text{-}28$ lipoproteins were virtually absent (Figure 20d).

After six weeks on the LARD-CS diet (Period 4) the HDL-I species again appeared as the main peak, though subpopulations of smaller particle size were observed as discrete peaks (Figure 20e). Progeny exhibited some $F^{o}_{1.20}9\text{-}28$ lipoprotein material, though not as much as was observed during Period 2 on the same diet. None of the patterns of the four progeny exhibited a distinct peak of $F^{o}_{1.20}9\text{-}28$ lipoprotein material during Period 4; this material appeared, rather, as a tail extending from the HDL-I peak.

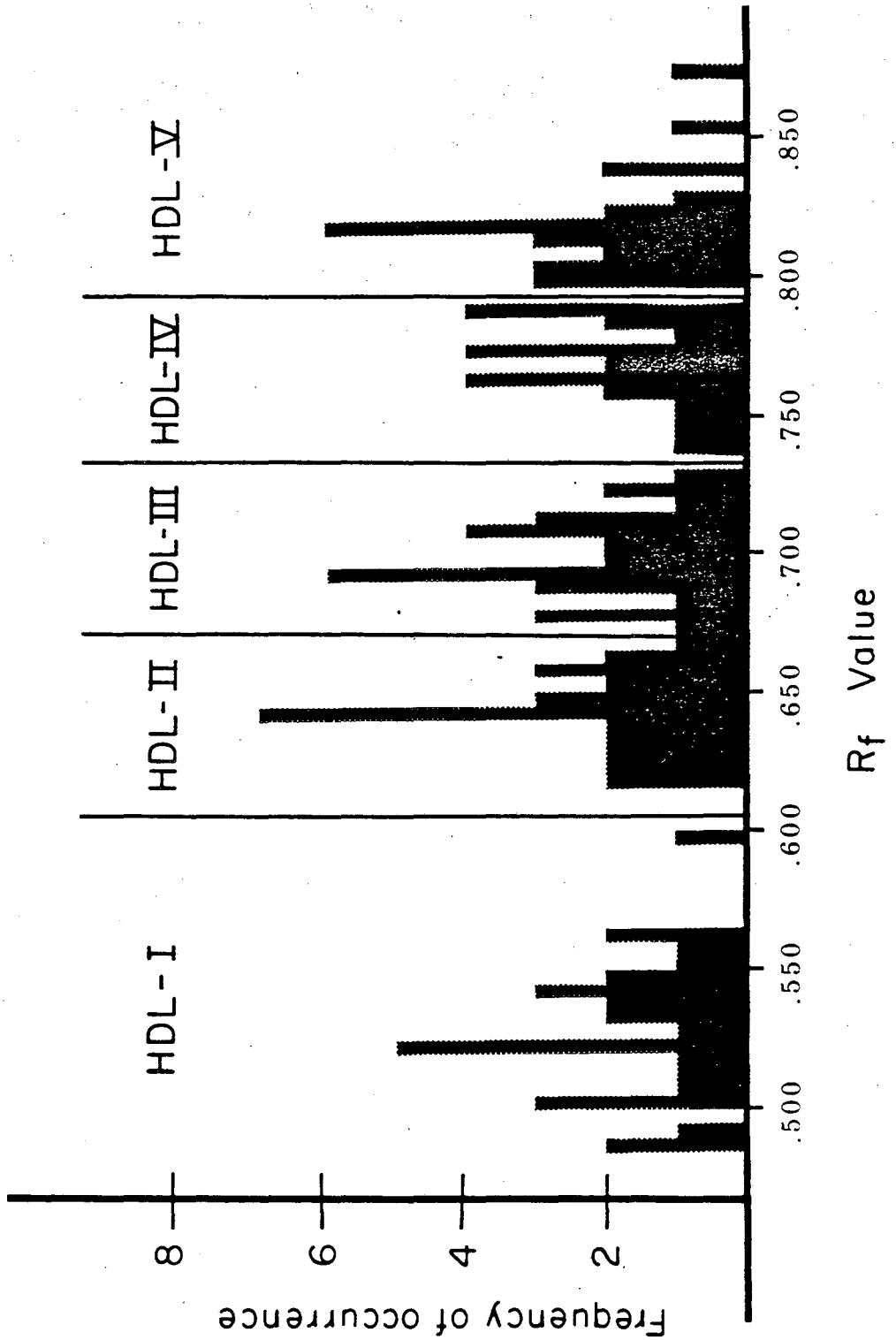
When the progeny were returned to the LARD+CS diet for the last six weeks of the study (Period 5), the HDL pattern

showed five distinct subpopulations. The $F^{o}_{1.20}$9-28 lipoprotein material appeared as one or two broad peaks within the 125-220A particle size range.

Although not all of the patterns of the progeny on all diets exhibited the same number of discrete HDL species, the peak particle sizes of such HDL species (when they could be determined) occurred within similar particle size intervals regardless of the diet they consumed. When the frequency of occurrence of peak HDL particle sizes from all baboons on all 3 diets was plotted as a function of R_f value (Figure 21), frequency maxima occurred within the same R_f intervals already established for baboon HDL-I, HDL-II, HDL-III, HDL-IV, and HDL-V (see Figure 2) for pedigreed baboons consuming only the LARD+CS diet. Hence, dietary manipulations in this study had little effect on the mean particle sizes of baboon HDL subpopulations, but, rather, affected the relative mass distribution among the HDL subpopulations.

Figure 21.

Frequency of occurrence of R_f values of HDL peaks as observed on 4-30% electrophoretic gradient gels. Electrophoresis was performed on the serum d≤1.20 g/ml fraction from all five progeny of sire 102 at the following time points in the diet sequence outlined in Table 3: LARD+CS diet (at least 1 year), LARD-CS diet (6 weeks), CHOW diet (6 weeks), CHOW diet (12 weeks), LARD-CS diet (6 weeks) and LARD+CS diet (6 weeks); n=30. The R_f intervals containing each of the five HDL subpopulations determined from this frquency plot compare well with those determined in Figure 2, for baboons consuming the LARD+CS diet.


HDL-I
HDL-II
HDL-III
HDL-IV
HDL-V
Frequency of occurrence
8
6
4
2
.500
.550
.600
.650
.700
.750
.800
.850
Rf Value

2. Lipoproteins within the Serum d<1.063 g/ml Fraction

2.1. Lipoprotein Concentration Measurements Made by Analytic Ultracentrifugation of the Serum d<1.063 g/ml Fraction

Table 21 lists the mean ± SD concentrations of LDL (S^{o}_{f}5-12), IDL (S^{o}_{f}12-20), and VLDL (S^{o}_{f}20-100) and LDL peak S^{o}_{f} rate of the four pedigreed progeny of sire 102 throughout the dietary sequence. During Period 1, this group of four progeny exhibited mean lipoprotein concentrations within the serum d≤1.063 g/ml fraction quite comparable to the means for all progeny of sire 102 (see Table 14). After cholesterol was removed from the diet for six weeks (LARD-CS diet, Period 2), LDL levels decreased by 49% ($P<0.005$, relative to the LARD+CS diet), as IDL decreased by 60% ($P<0.01$) and VLDL decreased by 90% ($P<0.1$).

After 12 weeks on the CHOW diet (Period 3), LDL decreased an additional 22%, relative to the previous diet (NS), IDL decreased by 79% ($P<0.1$) and VLDL was now completely absent from the serum. LDL peak flotation rate, though, decreased markedly from S^{o}_{f}8.76 on the LARD-CS diet to S^{o}_{f}6.06 on the CHOW diet ($P<0.005$).

When the progeny were placed on the LARD-CS diet for six weeks (Period 4), all lipoprotein measurements returned to essentially the same values observed previously on the LARD-CS diet (Period 2). That is, LDL (NS), IDL ($P<0.05$), and VLDL ($P<0.05$) concentrations and LDL peak S^{o}_{f} rate ($P<0.005$) each increased when the baboons were switched from

Table 21. Mean ± SD concentrations (mg/dl) of LDL, IDL and VLDL and the peak S^{o}_{f} rate (svedbergs) for four progeny of sire 102 during the sequence of dietary periods described in Table 3. All values were determined at the end of each diet period.

Period	Diet	LDL	IDL	VLDL	Peak S^{o}_{f} Rate
1	LARD+CS*	195 ± 47	47 ± 10	10 ± 10	8.48 ± .81
2	LARD-CS**	99 ± 27	19 ± 13	1 ± 2	8.76 ± .32
3	CHOW***	77 ± 26	4 ± 4	0 ± 0	6.06 ± .55
4	LARD-CS	104 ± 60	14 ± 8	2 ± 1	8.65 ± .38
5	LARD+CS	151 ± 97	71 ± 59	21 ± 21	9.29 ± .18

* LARD+CS diet, column 1 of Table 2

** LARD-CS diet, column 2 of Table 2

*** CHOW diet, column 3 of Table 2

the CHOW diet to the LARD-CS diet.

When cholesterol was added back to the diet for the last six weeks of the study (Period 5, LARD+CS diet), increases, relative to the LARD-CS diet, were observed in LDL (NS), IDL ($P<0.1$) and VLDL ($P<0.1$) concentrations and LDL peak flotation (S^{o}_{f}) rate ($P<0.05$). When these values, obtained during Period 5 (LARD+CS diet), were compared to those of Period 1 (LARD+CS diet), it appeared that LDL concentrations were slightly lower ($P=0.1$) and LDL peak flotation rates slightly faster ($P<0.1$) at the end of Period 5 than during Period 1.

2.2. Lipoprotein Size Distributions Measured by Gradient Gel Electrophoresis of the Serum d<1.063 g/ml Fraction

To evaluate whether the observed dietary cholesterol and fat dependence of the LDL peak S^{o}_{f} rate was due to changes in LDL particle size, the size distribution of lipoproteins within the serum $d \leq 1.063$ g/ml fraction was examined by gradient gel electrophoresis in 2-16% polyacrilamide gels. Table 22 lists mean $\pm$ SD LDL peak flotation rate (by analytic ultracentrifugation) and LDL peak particle sizes (by gradient gel electrophoresis) for all four progeny at two time points in Period 3 (six and twelve weeks on the CHOW diet following six weeks on the LARD-CS diet), at the end of Period 4 (six weeks on the LARD-CS diet), and at the end of Period 5 (six weeks on the LARD+CS diet). Mean LDL peak S^{o}_{f} rate values closely correlated with LDL peak

Table 22. Comparison of the mean ± SD of the LDL peak S^o_f rate (measured by analytic ultracentrifugation) and of the LDL peak particle diameter (measured by gradient gel electrophoresis) for four progeny of sire 102 during the sequence of dietary periods described in Table 3. Measurements were made after 6 and 12 weeks on the CHOW diet and after 6 weeks on each of the LARD-CS and LARD+CS diets.

Period	Diet	LDL Peak S^o_f Rate (s)	LDL Peak Particle Diameter (A)
3 (6 weeks)	CHOW	8.12 ± .41	274 ± 4
3 (12 weeks)	CHOW	6.06 ± .55[b]	267 ± 2[b]
4	LARD-CS	8.65 ± .38[a]	277 ± 4[c]
5	LARD+CS	9.29 ± .18[c]	281 ± 2[d]

Linear regression: Diameter(A) = 4.18 * S^o_f Rate(s) + 241A

(r = 0.99)

a: different from previous diet by P<0.005

b: different from previous diet by P<0.01

c: different from previous diet by P<0.05

d: different from previous diet by P<0.1

particle size values (correlation coefficient, r = .99) indicating a change of 4.2A in particle size for a change in peak flotation rate of 1 svedberg (S^{o}_{f} unit).

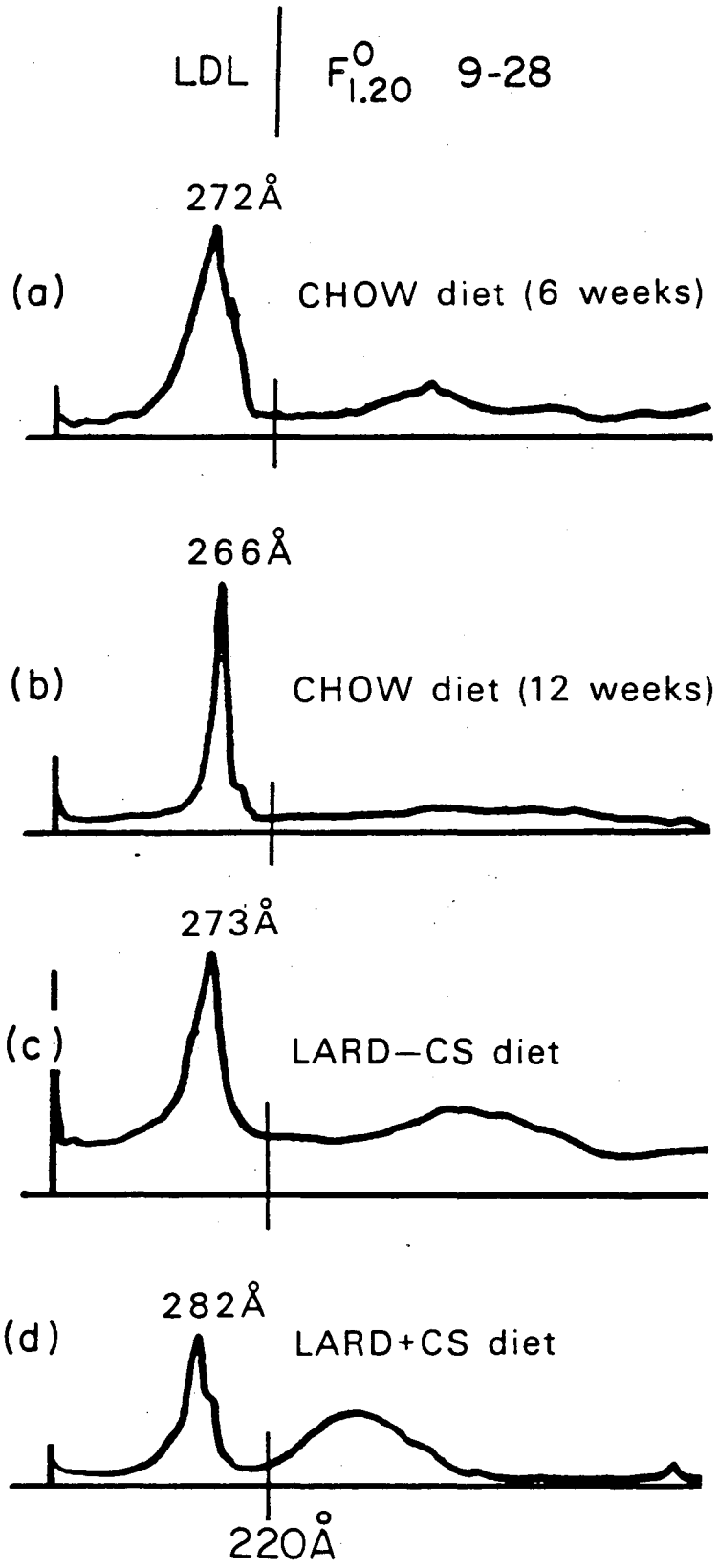
Figure 22 shows gradient gel electrophoretic scans of gels for one representative pedigreed progeny (baboon 2092) following the sequence of diets described in Table 22. After six weeks on the CHOW diet (Figure 22a), the LDL of all progeny appeared as a single major peak (peak particle size 270-278A) though all progeny also showed a small amount of lipoprotein material trailing into larger particle sizes. One progeny (2092) exhibited a minor peak of particle size 257A.

After twelve weeks on the CHOW diet (Figure 22b) the LDL again appeared as a single major peak, but of smaller particle size (range: 266-270A), consistent with the decrease in peak flotation rate observed at this time point (see Table 22). A minor peak corresponding to particles of smaller size (range: 249-254A) was also apparent in the gradient gel electrophoretic patterns of three of the four progeny. In contrast, larger sized material, observed after six weeks on the CHOW diet, was no longer seen after twelve weeks.

When saturated fat was added back to the diet for six weeks (LARD-CS diet, Period 4), the major LDL peak was now of larger particle size (range:273-282A), consistent with the observed increase in peak flotation rate (Table 22). In addition, some of the baboons exhibited material of larger

Figure 22.

Gradient gel electrophoretic patterns (2-16% gels) of the serum $d \leq 1.063$ g/ml fraction from one representative baboon (animal 2092) during the sequence of dietary periods outlined in Table 3. After 6 weeks on the CHOW diet (immediately following the LARD+CS and LARD-CS diets, respectively) (a), the LDL exhibits a broad peak of particle size 272A. After an additional 6 weeks on the CHOW diet (b), the LDL peak is of smaller particle size (266A). The particle size of the LDL peak increases (273A) when the baboon is consuming the LARD-CS diet for 6 weeks (c), and increases further (282A) after 6 weeks on the LARD+CS diet (d).


LDL | $F^{0}_{1.20}$ 9-28
272Å
(a)
CHOW diet (6 weeks)
266Å
(b)
CHOW diet (12 weeks)
273Å
(c)
LARD–CS diet
282Å
(d)
LARD+CS diet
220Å

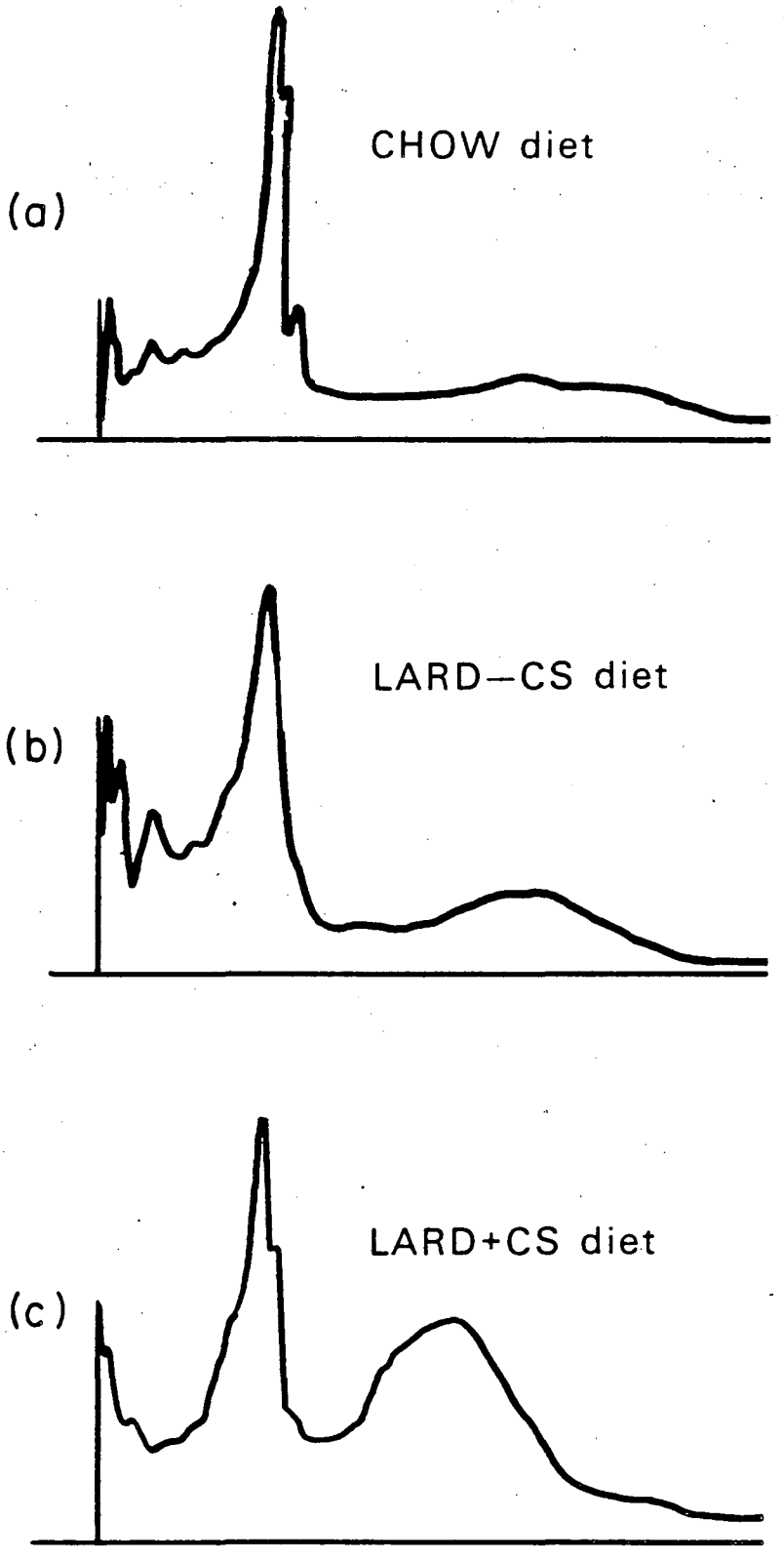
particle size trailing from the major LDL peak and some showed a broad peak of material within the 125-220A particle size range. These results are consistent with the observed presence of low concentrations of IDL and $F^{o}_{1.20}9\text{-}28$ lipoproteins, respectively, in these progeny when saturated fat was added to the diet (previous section, Tables 20 and 21).

When dietary cholesterol was added (LARD+CS diet, Period 5), the LDL peak in the gradient gel electrophoretic patterns shifted to larger particle size (range:278-284A) and demonstrated additional minor peaks indicative of material of both larger and smaller particle size, as well as the presence of $F^{o}_{1.20}9\text{-}28$ lipoproteins.

In order to obtain information about the size distributions of lipid-rich VLDL and IDL species in the baboon, serum fractions from the progeny at the end of Periods 3,4, and 5 were electrophoresed (2-16% gradient gel) and stained with lipophilic Oil Red O (Figure 23). Under these conditions, the lipid-rich VLDL and IDL lipoprotein species become more prominant on the gradient gel electrophoretic patterns. In general, two or more lipoprotein peaks were observed within the particle size range >290A, indicating polydispersity within the IDL and VLDL classes.

Figure 23.

Gradient gel electrophoretic patterns (2-16% gels) of the serum d≤1.063 g/ml fraction from one representative progeny (animal 2092) illustrating the polydispersity within the IDL and VLDL material (to the left of the major (LDL) peak) which is observed when gradient gels are stained with the lipophilic stain, Oil Red O.


(a)
CHOW diet
(b)
LARD—CS diet
(c)
LARD+CS diet

Chapter VI: Effects of Saturation of Dietary Fat on the Lipoproteins of Non-Pedigreed Baboons: COCO+CS and CORN+CS Diets - Experiment II

1. Experimental Design

Twenty-four unrelated baboons, ranging in age from 3 to 5 years were used as subjects. The baboons were fed, alternately, the COCO+CS (coconut oil with cholesterol) and CORN+CS (corn oil with cholesterol) diets for each of three six week periods. Serum samples obtained at the end of each six week period were utilized for all studies described in this chapter. More information on these baboons and on the design of Experiment II is provided in Chapter II, Sections 1.2 and 1.4.

2. Serum Total and Lipoprotein Cholesterol Concentrations Measured by Heparin-Manganese Chloride Precipitation

Measured values of the mean and 95% confidence limits of total serum cholesterol, as well as precipitable (by heparin-manganese chloride) and non-precipitable serum cholesterol (VLDL+LDL-C and HDL-C, respectively) for males and females on both the CORN+CS and COCO+CS diets are shown in Table 23. Relative to the CORN+CS diet, the COCO+CS diet significantly increased total serum cholesterol ($P<0.01$) by increasing both precipitable and non-precipitable cholesterol ($P<0.001$ and $P<0.01$, respectively). Although there was no significant sex difference in any cholesterol measurement, there was a significant diet-by-sex interaction on HDL-C ($P<0.05$); that is, the COCO+CS diet induced a greater elevation in HDL-C in males (77 mg/dl on CORN+CS vs 130 mg/dl on COCO+CS diet) than was observed in females (74 mg/dl vs 130 mg/dl).

Table 24 shows the effects of diet, sex and diet-by-sex interactions as multiplicative effects, as defined in Methods. In particular, total cholesterol is shown to be, on average, 43% higher on the COCO+CS diet relative to the CORN+CS diet (diet effect of 1.43). This increase is associated with a 35% increase in precipitable cholesterol and a 58% increase in non-precipitable cholesterol (all increases $P<0.01$).

3. Lipoprotein Concentrations Measured by Analytic Ultracentrifugation

3.1. Concentrations of Lipoproteins within the Serum d<1.063 g/ml Fraction

The concentrations of lipoprotein fractions within the serum d≤1.063 g/ml fraction are shown in Table 23, and the corresponding multiplicative diet and sex effects are listed in Table 24. LDL, measured at d 1.063 g/ml as material within the $S^{o}_{f}5-12$ flotation interval, were slightly but not significantly higher on the COCO+CS diet. However, there was a sex difference with respect to LDL concentrations; namely, females possessed higher concentrations of $S^{o}_{f}5-12$ lipoproteins on both diets. IDL ($S^{o}_{f}12-20$) and VLDL ($S^{o}_{f}20-100$) concentrations were, in fact, significantly lower on the COCO+CS diet ($P<0.05$). These saturated fat fat-induced reductions in both IDL and VLDL were greater in males than females (diet-by-sex interactions =0.47 and 0.28, respectively, $P<0.01$).

Saturated fat (the COCO+CS diet) induced significantly higher levels of lipoproteins in the serum d≤1.063 g/ml fraction measured within the $S^{o}_{f}0-5$ flotation interval. This lipoprotein species was increased, on the average, from 10mg/dl when the animals consumed the CORN+CS diet to 106 mg/dl when they were fed the COCO+CS diet ($P<0.01$). In addition, this saturated fat-induced increase was considerably greater in males than in females (diet-by-sex

Table 23--Mean total, VLDL+LDL, and HDL cholesterol concentrations (n = 24), and ultracentrifugal lipoprotein fraction concentrations (n = 12) (mg/dl) in serum by type of dietary fat and sex.

Variable	Polyunsaturated fat (corn oil) with cholesterol				Saturated fat (coconut oil) with cholesterol			
	Male		Female		Male		Female	
	Mean	95% confidence limits	Mean	95% confidence limits	Mean	95% confidence limits	Mean	95% confidence limits
Total cholesterol	159	145-174	149	136-164	231	211-253	211	193-232
Lipoprotein cholesterol by precipitation								
VLDL+LDL cholesterol	77	64-92	71	59-85	99	82-118	101	84-121
HDL cholesterol	77	69-85	74	66-82	130	117-145	108	97-120
Analytical ultracentrifugation at density								
d 1.063 g/ml								
S_f° 0-5 ($F^\circ_{1.20}$ 9-28 lipoproteins)	5	2-12	16	6-45	151	59-391	62	21-180
S_f° 5-12 (LDL)	127	91-177	179	124-259	135	97-188	258	179-372
S_f° 12-20 (IDL)	64	32-126	46	22-96	10	5-20	33	16-70
S_f° 20-400 (VLDL)	25	8-71	13	4-42	0	0-1	5	1-18
d 1.20 g/ml								
$F^\circ_{1.20}$ 0-2 (HDL-III - HDL-V)+	50	39-63	46	36-58	43	34-55	42	33-53
$F^\circ_{1.20}$ 2-4 (HDL-II)+	133	115-153	115	100-132	127	110-146	109	95-126
$F^\circ_{1.20}$ 4-9 (HDL-I)+	199	162-244	235	191-288	290	236-356	328	267-404
$F^\circ_{1.20}$ 9-28	4	2-9	11	5-23	154	78-305	72	36-143
$F^\circ_{1.20}$ 9-14	1	0-4	7	2-20	70	39-125	58	32-104
$F^\circ_{1.20}$ 14-20	0*	-	0	0-1	42	13-131	8	2-26
$F^\circ_{1.20}$ 20-28	0*	-	0*	-	19	2-122	1	0-9

*All observed values zero

+See Methods for definition of HDL subpopulations.

Table 24--Estimated diet, sex, and diet-by-sex interaction effects* on total and lipoprotein cholesterol (n = 24) and on lipoprotein fraction concentrations (n = 12).

Variable	Fat effect		Sex effect		Fat-by-sex interaction	
	Effect	95% confidence limits	Effect	95% confidence limits	Interaction effect	95% confidence limits
Total cholesterol	1.43	1.35-1.53	1.08	0.95-1.22	1.01	0.98-1.04
Lipoprotein cholesterol by precipitation						
VLDL+LDL cholesterol	1.35	1.16-1.58	1.03	0.81-1.30	0.95	0.88-1.03
HDL cholesterol	1.58	1.43-1.75	1.11	0.97-1.26	1.07	1.02-1.13
Analytical ultracentrifugation by density						
d 1.063 g/ml						
S_f° 0-5 ($F_{1.20}^\circ$ 9-28 lipoproteins)	10.95	2.73-43.88	0.85	0.31-2.28	2.87	1.42-5.79
S_f° 5-12 (LDL)	1.24	0.72-2.11	0.61	0.46-0.81	0.86	0.66-1.13
S_f° 12-20 (IDL)	0.34	0.12-0.98	0.65	0.35-1.21	0.47	0.27-0.80
S_f° 20-400 (VLDL)	0.13	0.02-0.64	0.51	0.22-1.21	0.28	0.12-0.63
d 1.20 g/ml						
$F_{1.20}^\circ$ 0-2 (HDL-III - HDL-V)+	0.89	0.69-1.14	1.05	0.82-1.35	0.97	0.76-1.25
$F_{1.20}^\circ$ 2-4 (HDL-II)+	0.95	0.82-1.10	1.16	1.00-1.34	1.00	0.86-1.16
$F_{1.20}^\circ$ 4-9 (HDL-I)+	1.43	1.15-1.77	0.86	0.70-1.07	1.02	0.82-1.27
$F_{1.20}^\circ$ 9-28	13.36	6.57-27.19	0.95	0.47-1.94	2.23	1.10-4.54

*Effect is statistically significant ($P < 0.05$) if 95% confidence limits do not include 1.00.

+See Methods for definition of HDL subpopulations.

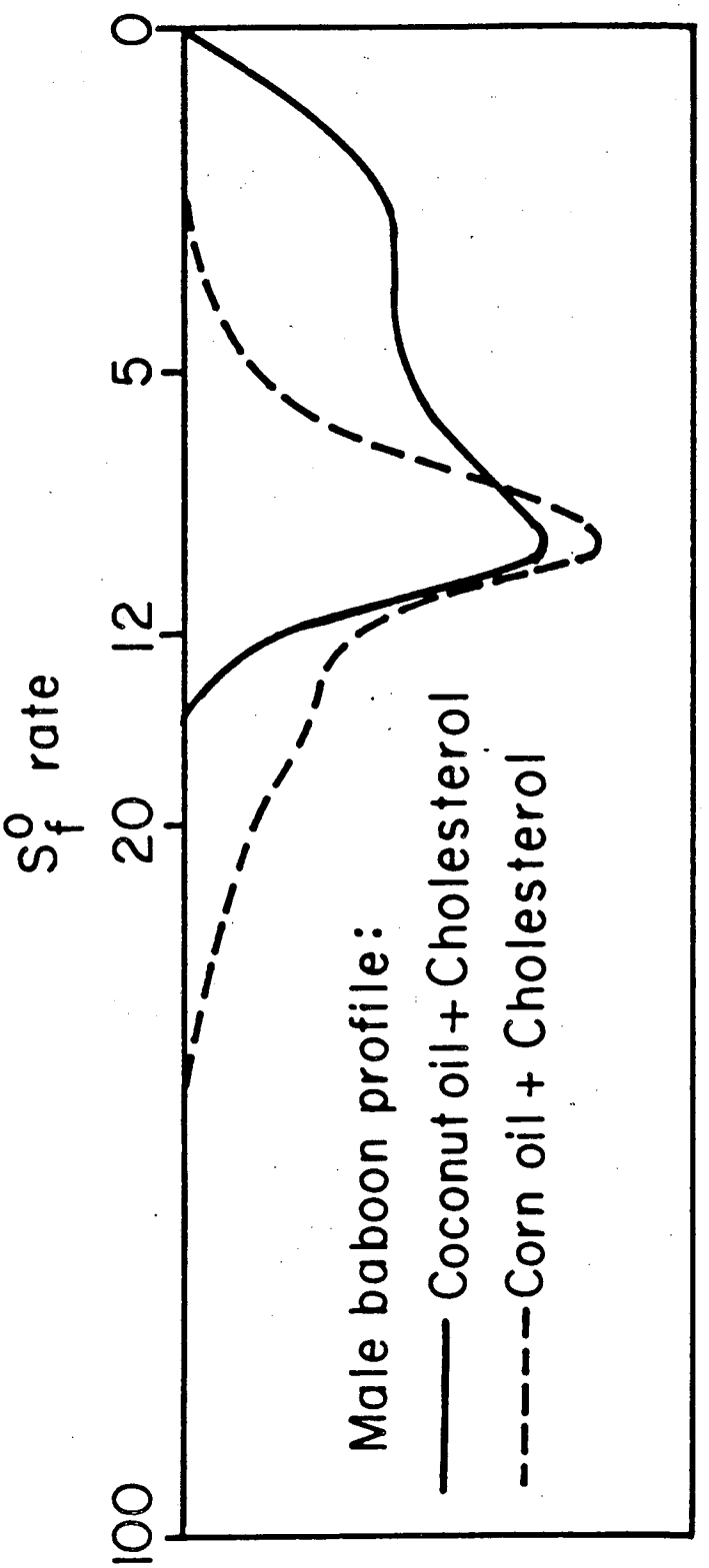
interaction, $P<0.01$). The lipoprotein species measured within the $S^{o}_{f}0-5$ flotation interval correspond to the $F^{o}_{1.20}9-28$ lipoproteins characterized in Chapter 2 of this dissertation. Additional information on the effect of dietary fat on these lipoproteins, as well as preliminary identification of subpopulations within this species are given later in this chapter (Sections 3.2 and 5).

Figure 24 shows the effect of dietary fat on the analytic ultracentrifugal schlieren pattern of the serum $d \leq 1.063$ g/ml fraction from a representative male baboon (animal number 1665). The LDL of this animal, when consuming either diet, appeared as a single peak floating within the $S^{o}_{f}5-12$ interval (peak flotation rate on COCO+CS, $S^{o}_{f}9.5$; on CORN+CS $S^{o}_{f}8.8$). The broad peak within the $S^{o}_{f}0-5$ flotation interval in the baboon when fed the COCO+CS diet represents $F^{o}_{1.20}9-28$ lipoproteins with a peak flotation rate of $S^{o}_{f}1.5$ for the animal shown in figure 24. Very little material appears within this interval when the same animal is consuming the CORN+CS diet.

On the other hand, saturated fat considerably reduced both IDL ($S^{o}_{f}12-20$) and VLDL ($S^{o}_{f}20-100$) in this baboon. In the typical female pattern (Figure 25), LDL levels are higher on both diets. In addition, $F^{o}_{1.20}9-28$, IDL and VLDL changes in response to diet are less pronounced than in the male pattern.

Figure 24.

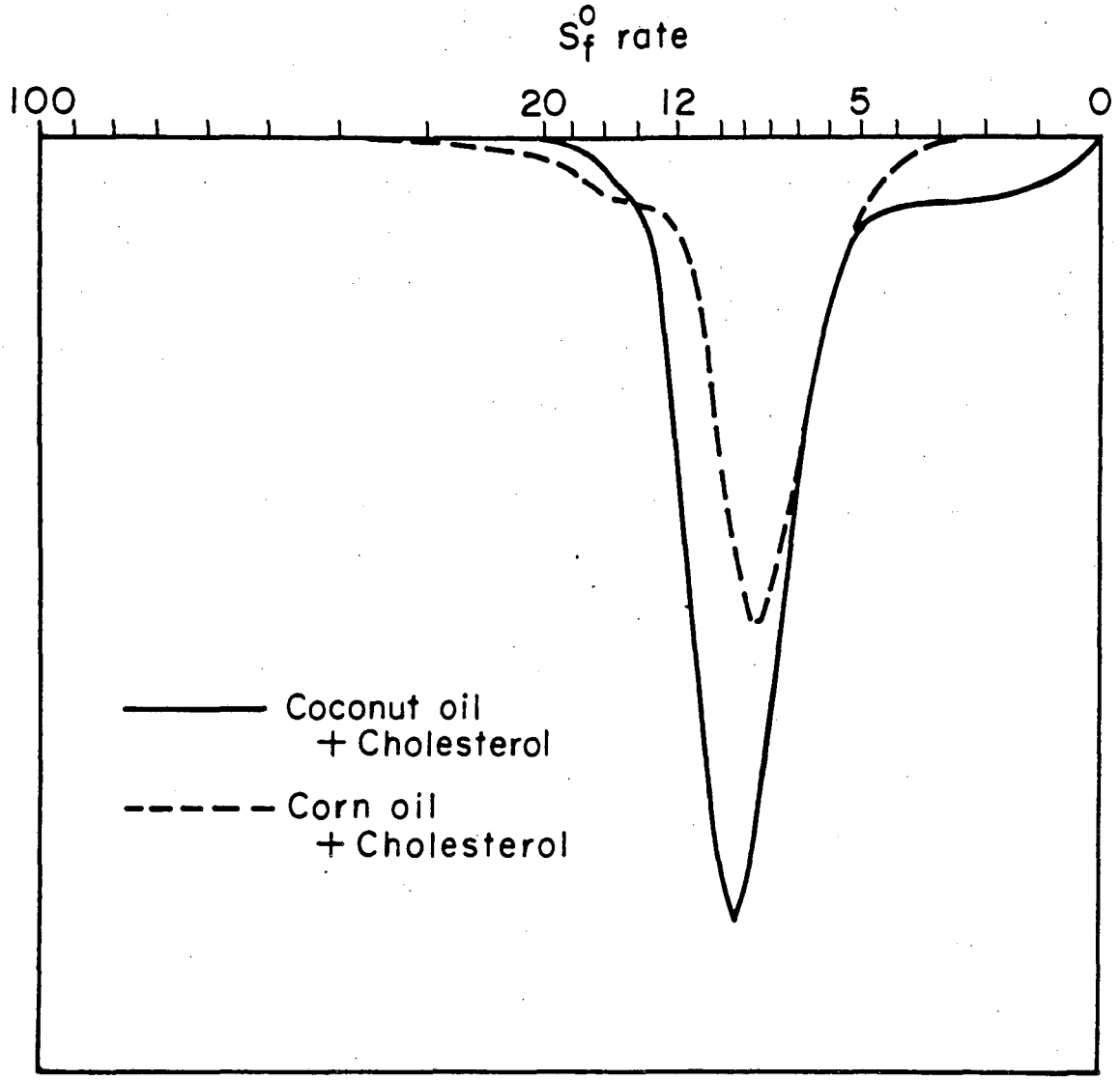
Typical dietary responses observed among male baboons illustrated by schlieren patterns of the serum $d \leq 1.063$ g/ml fraction from baboon 1665 after six week periods each on the coconut oil (saturated fat) plus cholesterol diet (——) and on the corn oil (polyunsaturated fat) plus cholesterol diet (---). For the animal shown, saturated fat elevated $F^{o}_{1.20}9\text{-}28$ lipoproteins ($S^{o}_{f}0\text{-}5$, 12 mg/dl on the corn oil diet vs 116 mg/dl on the coconut oil diet), did not affect LDL ($S^{o}_{f}5\text{-}12$, 136 mg/dl on the corn oil diet vs 144 mg/dl on the coconut oil diet), but decreased both IDL ($S^{o}_{f}12\text{-}20$, 47 mg/dl on the corn oil diet vs 7 mg/dl on the coconut oil diet) and VLDL ($S^{o}_{f}20\text{-}100$, 17 mg /dl on the corn oil diet vs 0 mg/dl on the coconut oil diet).


0
5
12
20
100
S_f^0 rate
Male baboon profile:
Coconut oil + Cholesterol
Corn oil + Cholesterol

XBL843-7627

Figure 25.

Typical dietary response observed among female baboons illustrated by schlieren patterns of the serum $d \leq 1.063$ g/ml fraction from baboon 3076 after six week periods each on the coconut oil (saturated fat) plus cholesterol diet (——) and on the corn oil (polyunsaturated fat) diet (---). For the animal shown, saturated fat elevated $F^{o}_{1.20}$9-28 lipoproteins (S^{o}_{f}0-5, 10 mg/dl on the corn oil diet vs 55 mg/dl on the coconut oil diet), increased LDL (S^{o}_{f}5-12, 204 mg/dl on the corn oil diet vs 340 mg/dl on the coconut oil diet) and had little effect on either IDL (S^{o}_{f}12-20, 30 mg/dl on the corn oil diet vs 29 mg/dl on the coconut oil diet) or VLDL (S^{o}_{f}20-100, 5 mg/dl on the corn oil diet vs 0 mg/dl on the coconut oil diet).


S_f^0 rate
100
20
12
5
0
Coconut oil
+ Cholesterol
Corn oil
+ Cholesterol

XBL843-7599

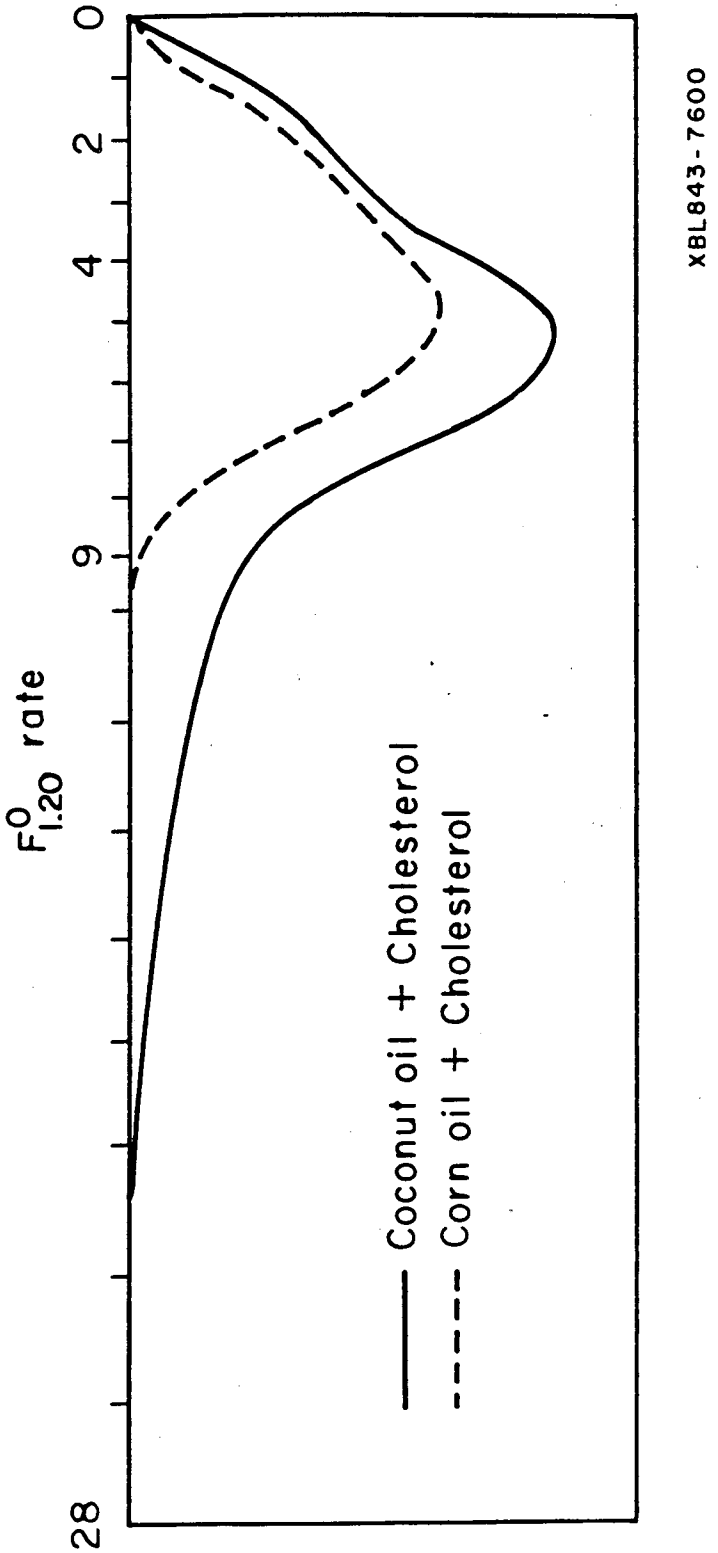
3.2. Concentrations of HDL and $F^{o}_{1.20}9\text{-}28$ Lipoproteins within the Serum d<1.20 g/ml Fraction

The COCO+CS diet induced significantly higher concentrations of the larger size (>100A) apoA-I-containing lipoprotein species (i.e. $F^{o}_{1.20}9\text{-}28$ lipoproteins and HDL-I) but did not affect concentrations of the smaller HDL subpopulations (see Methods and Chapter III for the biochemical definition of the HDL subpopulations in the baboon). As shown in Tables 23 and 24, material within the $F^{o}_{1.20}4\text{-}9$ flotation interval (approximating the HDL-I subpopulation) was 43% higher (217 mg/dl vs 309 mg/dl), on average, when the animals were fed the COCO+CS diet. In addition, $F^{o}_{1.20}9\text{-}28$ lipoproteins were higher (8 mg/dl vs 113 mg/dl) when the animals were fed the saturated fat diet. Most of this $F^{o}_{1.20}9\text{-}28$ lipoprotein material was observed within the the $F^{o}_{1.20}9\text{-}14$ interval, corresponding to the smaller, denser species within the $F^{o}_{1.20}9\text{-}28$ lipoprotein spectrum (78). There was a significant diet-by sex interaction for $F^{o}_{1.20}9\text{-}28$ lipoproteins because males demonstrated a much greater increase in these lipoproteins on the saturated fat diet than females. Saturated fat, however, had no significant effect on any other HDL subpopulations (HDL-II, measured as material within the $F^{o}_{1.20}2\text{-}4$ flotation interval; and HDL-III through HDL-V, measured as material within the $F^{o}_{1.20}0\text{-}2$ flotation interval).

Figure 26 illustrates the effect of the saturation of dietary fat on the schlieren pattern of the d≤1.20 g/ml

Figure 26.

Schlieren patterns of the $F^{o}_{1.20}9\text{-}28$ lipoproteins in the serum $d \leq 1.20$ g/ml fraction from a representative baboon (animal 1659) after six week periods each on the coconut oil (saturated fat) plus cholesterol diet (——) and the corn oil (polyunsaturated fat) plus cholesterol diet (---). The patterns from this baboon demonstrate saturated fat-induced elevations in $F^{o}_{1.20}9\text{-}28$ lipoproteins (<10 mg/dl on the corn oil diet vs 101 mg/dl on the coconut oil diet) and HDL-I ($F^{o}_{1.20}4\text{-}9$, 194 mg/dl on the corn oil diet vs 325 mg/dl on the coconut oil diet).


0
2
4
9
28
$F^{0}_{1.20}$ rate
Coconut oil + Cholesterol
Corn oil + Cholesterol
XBL843-7600

fraction of a typical animal in this experiment (1659). When consuming the corn oil diet, all the lipoprotein material (HDL) is observed within the $F^{o}_{1.20}0\text{-}9$ flotation interval. When the same animal is consuming the COCO+CS diet, the faster floating HDL species (HDL-I within the $F^{o}_{1.20}4\text{-}9$ interval) are considerably increased, and material is also apparent within the $F^{o}_{1.20}9\text{-}28$ flotation interval.

4. Lipoprotein Size Distributions by Gradient Gel Electrophoresis

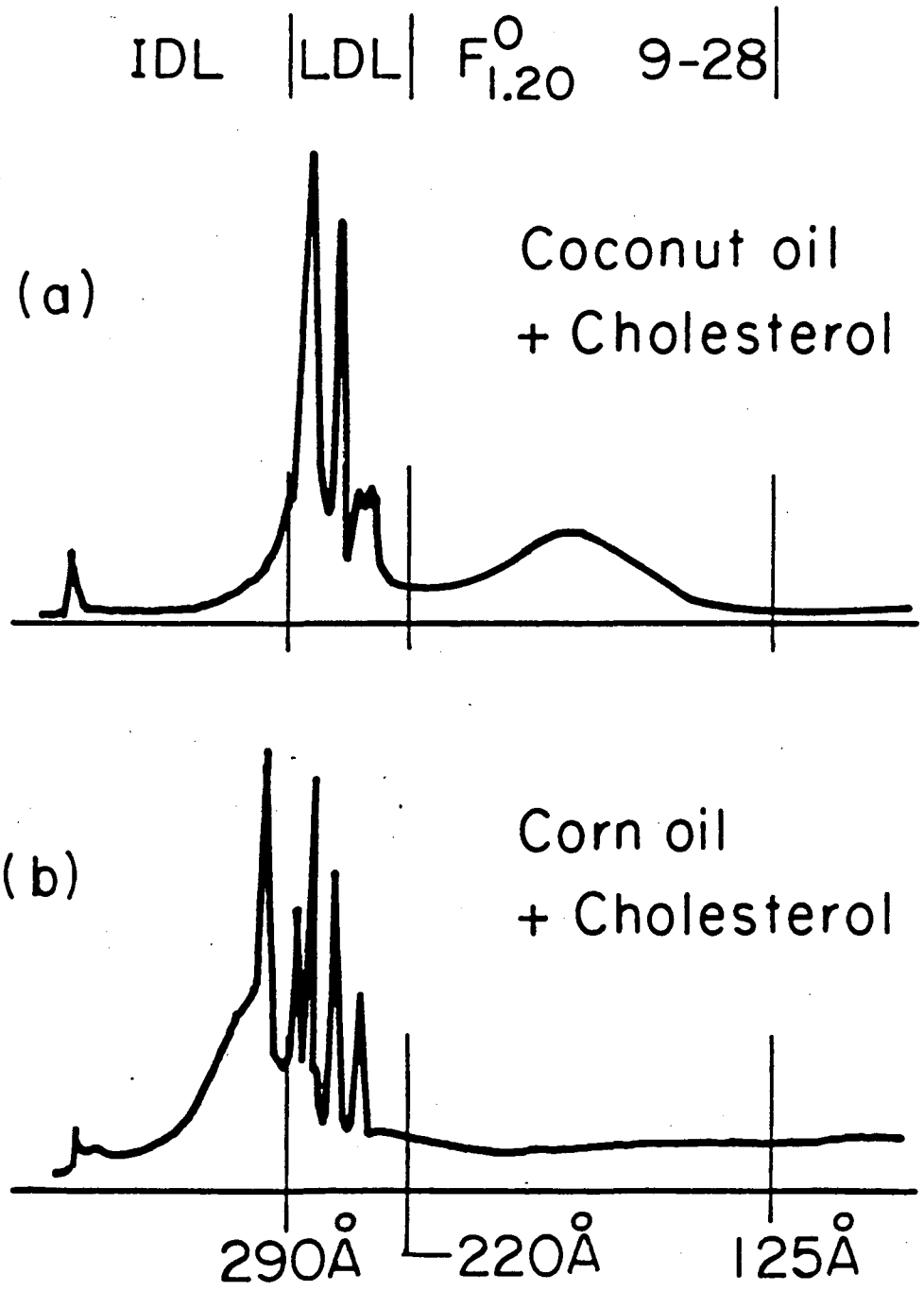
4.1. LDL and IDL Size Distributions

The particle size distribution of material isolated within the serum d≤1.063 g/ml fraction, obtained from all baboons while consuming both diets, was examined by gradient gel electrophoresis. Figure 27 compares the gradient gel electrophoresis profiles of lipoproteins in the serum d≤1.063 g/ml fraction from one animal (3081) consuming either the COCO+CS (Figure 27a) or CORN+CS (Figure 27b) diet. While consuming the COCO+CS diet, LDL material appeared as 4 distinct peaks within the 220-290A particle size range and $F^{o}_{1.20}$9-28 lipoproteins appeared as a broad peak within the 125-220A size range. When the same animal was fed the CORN+CS diet (Figure 27b), the LDL material retained its polydisperse character, while the $F^{o}_{1.20}$9-28 lipoprotein material was absent. Furthemore, while consuming the corn oil diet, IDL material was observed as polydisperse material of particle size >290A.

All animals on either diet usually exhibited two or more major peaks within the 220-290A size range. In Chapter III it was shown that this material has a hydrated density of 1.025-1.040 g/ml, contains mainly apoB as its protein moiety, and corresponds to the LDL material observed by analytic ultracentrifugation within the S^{o}_{f}5-12 flotation interval.

Figure 27

Comparison of gradient gel electrophoretic profiles of lipoproteins in the serum d≤1.063 g/ml fraction (electrophoresed on a 2-16% gradient gel) from baboon 3081 after six weeks on (a) the coconut oil (saturated fat) plus cholesterol diet and (b) the corn oil (polyunsaturated fat) plus cholesterol diet. This animal was selected to illustrate the appearance of IDL material on gradient gel electrophoretic profiles. In this case, there was an unusually high concentration of IDL and VLDL on the corn oil diet as determined by analytic ultracentrifugation (S^{o}_{f}12-100, 190 mg/dl), but a typical concentration of this material (S^{o}_{f}12-100, 36 mg/dl) on the coconut oil diet.


IDL
LDL
$F^{0}_{1.20}$
9-28
(a)
Coconut oil
+ Cholesterol
(b)
Corn oil
+ Cholesterol
290Å
220Å
125Å

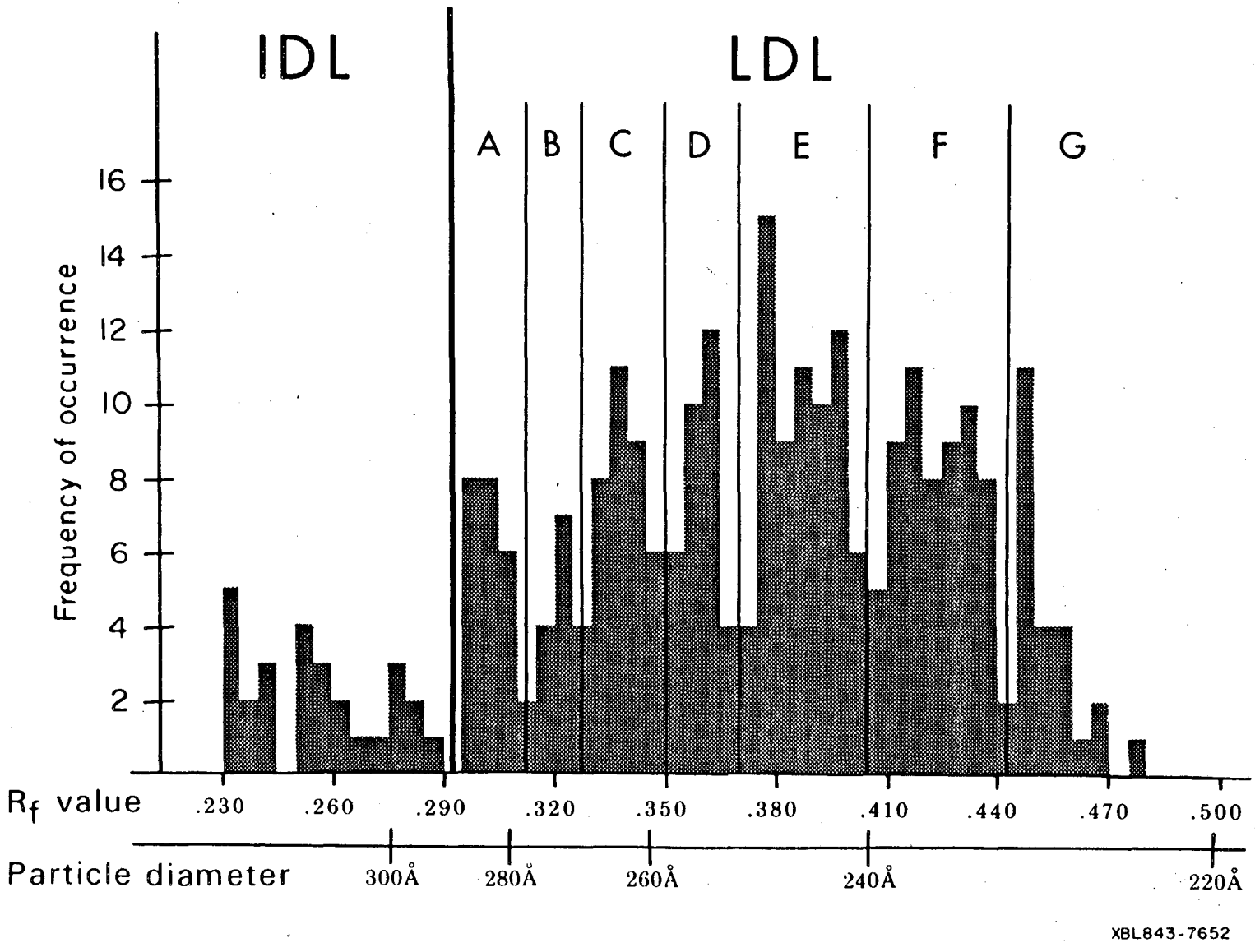
XBL843-7601

To determine if the discrete LDL peaks observed in the gradient gel electrophoretic patterns of most baboons represented distinct LDL subpopulations, the R_f value (ratio of the peak migration distance in the 2-16% gradient gel relative to the migration distance of an apoferritin standard in the same gel) of each LDL peak was tabulated and plotted as a histogram (Figure 28). The resulting frequency distribution indicated seven discrete modes between R_f .295-.500 (approximate particle diameter 220-290A) which have been designated A through G from largest to smallest particle size. Since the R_f intervals containing these modes were similar for both diets, the frequency distribution in Figure 28 includes observations from patterns for baboons on both diets.

Support for the concept that these modes represent distinct subpopulations of LDL was the observation that when the gradient gel electrophoretic pattern of an individual baboon possessed several peaks, each peak corresponded to a different mode, as designated in Figure 28. IDL material (of particle diameter >290A or R_f <.295) appeared as one or more peaks in the gradient gel electrophoretic patterns of baboons with elevated IDL (S^o_f12-20) and VLDL (S^o_f20-100) levels, usually while consuming the CORN+CS diet.

Figure 28

Frequency of observation of peaks at specific R_f values in gradient gel electrophoretic profiles of baboon LDL and IDL (derived from the serum d≤1.063 g/ml fraction and electrophoresed in a 2-16% gel) based on combined data from baboons on both the coconut oil and corn oil diets (n=72). The frequency distribution indicates seven modes within the LDL particle size range (which are designated A through G), supporting the concept that discrete peaks on gradient gel electrophoretic profiles represent distinct LDL subpopulations. Polydispersity was also apparent within the IDL particle size range (>290A). Approximate particle sizes are indicated at the bottom of the histogram.


IDL
LDL
A
B
C
D
E
F
G
16
14
12
10
8
6
4
2
Frequency of occurrence
R_f value
.230
.260
.290
.320
.350
.380
.410
.440
.470
.500
Particle diameter
300Å
280Å
260Å
240Å
220Å
XBL843-7652

4.2. $F^{o}_{1.20}9\text{-}28$ Lipoprotein Size Distributions

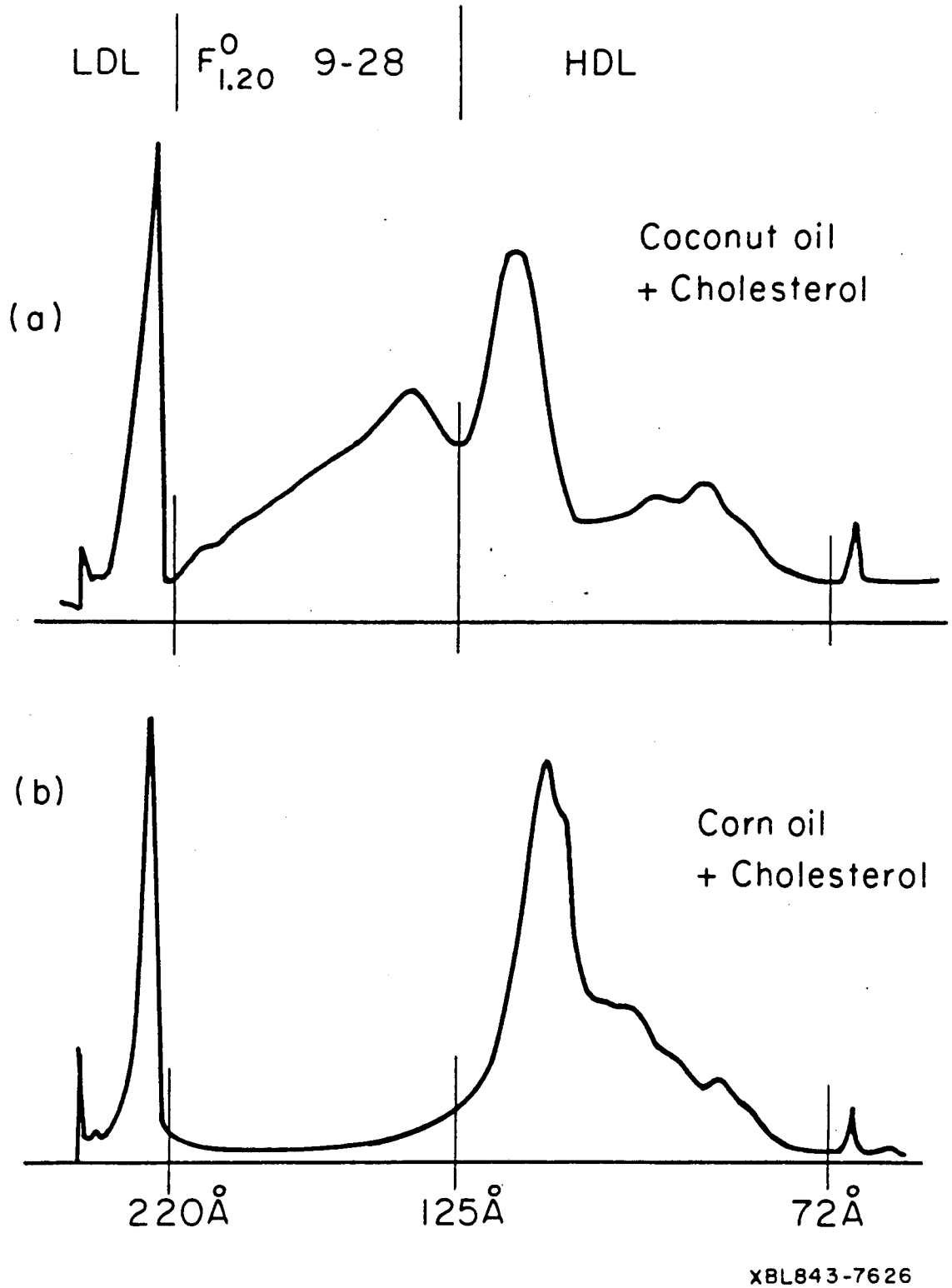
By gradient gel electrophoresis, $F^{o}_{1.20}9\text{-}28$ lipoproteins in baboons on the COCO+CS diet formed a broad distribution within the 125-220A particle size range. Figure 27 compares the electrophoretic patterns (2-16% gradient gel) of the serum $d \leq 1.063$ g/ml fraction from one baboon on the two different diets. The $F^{o}_{1.20}9\text{-}28$ lipoproteins lacked the distinct peaks which would indicate discrete particle subpopulations. The particle size distribution of $F^{o}_{1.20}9\text{-}28$ lipoprotein material and its appearance usually as a non-discrete continuum of material, in the sera of baboons on the COCO+CS diet, are characteristics similar to those reported for $F^{o}_{1.20}9\text{-}28$ lipoprotein material of baboons on the LARD+CS (see Figures 1 and 6 for examples of $F^{o}_{1.20}9\text{-}28$ lipoproteins appearing on 4-30% gradient gels, and Figures 7,8, and 12 for examples on 2-16% gels). As illustrated in Figure 27b, essentially no material is apparent within the $F^{o}_{1.20}9\text{-}28$ lipoprotein particle size range (125-220A) when the baboon is fed the CORN+CS diet.

4.3. HDL Size Distributions

The HDL size distribution was evaluated following gradient gel electrophoresis of the serum $d \leq 1.20$ g/ml fraction, obtained from all baboons on both diets. Figure 29 compares the gradient gel electrophoresis profile of lipoproteins in the serum $d \leq 1.20$ g/ml fraction from one representative baboon (animal 3080) exhibiting on the COCO+CS diet both HDL

Figure 29.

Comparison of gradient gel electrophoretic profiles of lipoproteins in the serum $d \leq 1.20$ g/ml fraction (electrophoresed on a 4-30% gel) from baboon 3080 after six weeks on (a) the coconut oil (saturated fat) plus cholesterol diet and on (b) the corn oil (polyunsaturated fat) plus cholesterol diet. Discrete subpopulations are apparent within the HDL particle size range (72-125A) on both diets; considerable $F^{o}_{1.20}$9-28 lipoprotein material (particle size range 125-220A) is seen only on the saturated fat diet (a).

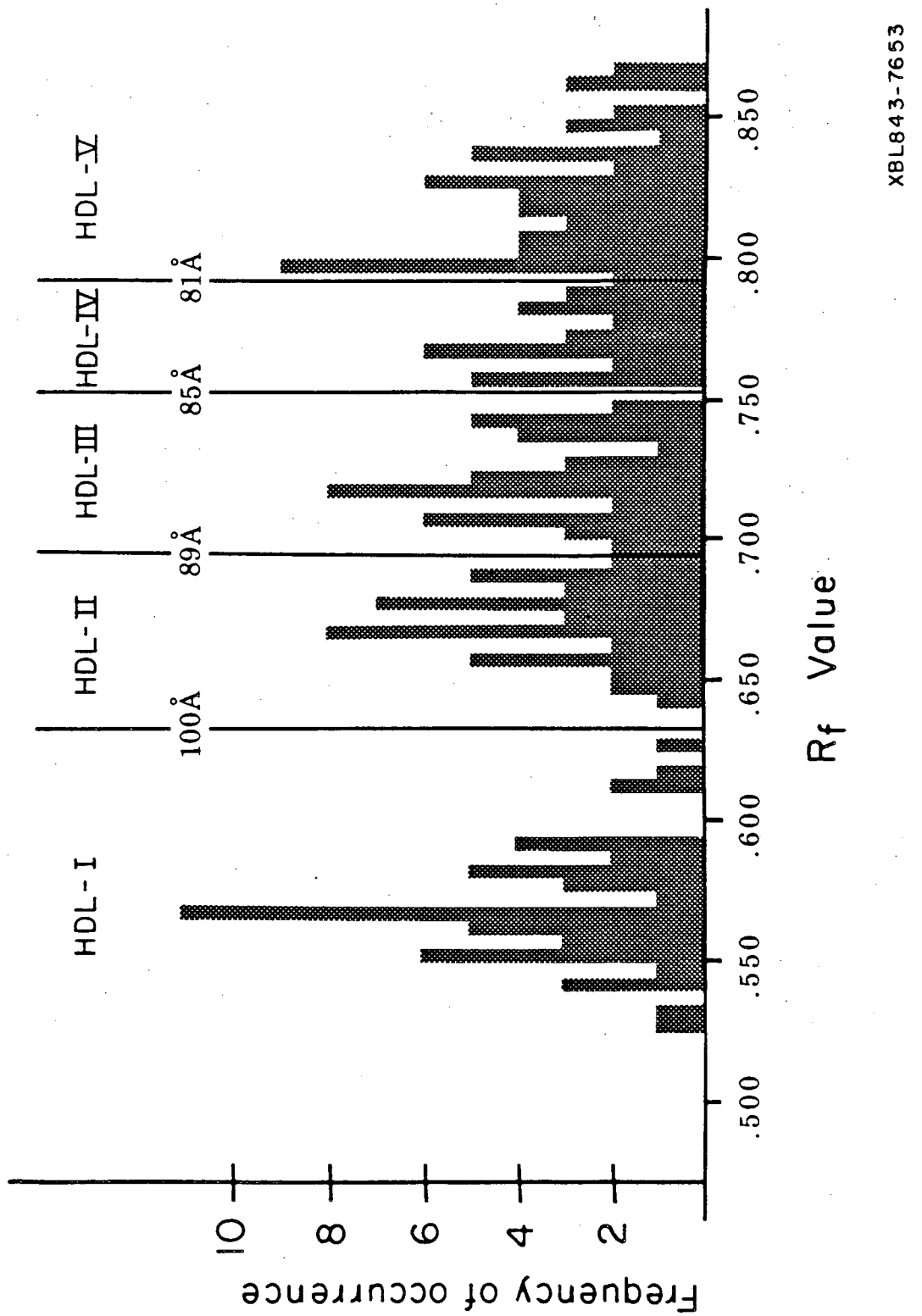

LDL
$F^{0}_{1.20}$ 9-28
HDL
(a)
Coconut oil
+ Cholesterol
(b)
Corn oil
+ Cholesterol
220Å
125Å
72Å
XBL843-7626

(particle size range 72-125A) and $F^{o}_{1.20}9$-28 lipoprotein (particle size range 125-220A) (Figure 29a), but only HDL material while consuming the CORN+CS diet (Figure 29b).

The major part of HDL in most baboons on both diets was observed as a single peak located within the 100-125A size range (mean $\pm$ SD, 108 $\pm$ 2A on the COCO+CS diet and 104 $\pm$ 2A on the CORN+CS diet). The remaining material was distributed among 3 to 4 peaks in the size range 72-100A. When the frequency of observation of individual peaks was plotted versus their peak R_f value (ratio of the peak migration distance in the 4-30% gradient gel relative to a bovine serum albumin standard in the same gel), distinct distribution maxima (Figure 30) occurred within specific size intervals. The R_f intervals containing the frequency maxima were used to specify baboon HDL subpopulations (designated HDL-I through HDL-V) and were found to be similar for baboons on both diets. The intervals compared well with the particle size ranges identifying baboon HDL subpopulations on other diets (Compare with Figures 2 and 21).

Figure 30.

Frequency of observation of peaks at specific R_f values in gradient gel electrophoretic profiles of baboon HDL (derived from the serum d≤1.20 g/ml fraction electrophoresed in a 4-30% gel) based on the combined data from baboons on both the coconut oil and corn oil diets (n=44). The frequency distribution indicates five modes within the HDL particle size range (which are designated HDL-I through HDL-V), supporting the concept that discrete peaks on a gradient gel electrophoretic profile represent distinct HDL subpopulations. Particle sizes at the boundaries between subpopulations are indicated.


HDL-I
HDL-II
HDL-III
HDL-IV
HDL-V
100Å
89Å
85Å
81Å
Frequency of occurrence
2
4
6
8
10
.500
.550
.600
.650
.700
.750
.800
.850
Rf Value

XBL843-7653

5. Quantification of Baboon Lipoproteins by Precipitation with Heparin-Manganese Chloride

The extent of precipitation of baboon lipoprotein classes in nine baboons was examined by comparing the ultracentrifugal schlieren patterns of the total serum lipoproteins with those of the non-precipitable lipoproteins. VLDL, IDL, and LDL were completely precipitated; however, precipitation of the $F^{o}_{1.20}9\text{-}28$ lipoprotein material was partial and was related to the flotation rate of the material within the $F^{o}_{1.20}9\text{-}28$ flotation interval. That is, the concentration of material within the $F^{o}_{1.20}9\text{-}14$ flotation interval was essentially unaffected by reaction with heparin-manganese chloride. In contrast, material within the $F^{o}_{1.20}14\text{-}20$ interval was reduced by 28%, on average; and material within the $F^{o}_{1.20}20\text{-}28$ interval showed an average reduction of 75%. The precipitability of material within the $F^{o}_{1.20}14\text{-}28$ interval would be consistent with the presence of apoE or apoB in the protein moiety of this material (Figure 10). Incomplete precipitability suggests either inadequate concentrations of reagents or possibly the presence of subpopulations within the $F^{o}_{1.20}9\text{-}28$ interval with different contents of apolipoproteins reactive with the heparin-manganese chloride reagent.

6. Individual Differences in the Profiles of $F^{o}_{1.20}9\text{-}28$ Lipoproteins

Two of the twelve baboons examined by both gradient gel electrophoresis and analytic ultracentrifugation showed patterns of $F^{o}_{1.20}9\text{-}28$ lipoproteins different from those of the others. Figure 31a compares the $d \leq 1.20$ g/ml lipoprotein profile of a typical animal (1664) and that of one of the two unusual animals (1478). Although the two animals shown had similar $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations, baboon 1664 had 42% of its $F^{o}_{1.20}9\text{-}28$ lipoproteins within the $F^{o}_{1.20}9\text{-}14$ subinterval and 20% in the $F^{o}_{1.20}20\text{-}28$ subinterval, while baboon 1478 had only 28% in the former subinterval and 43% in the latter subinterval.

In an attempt to resolve further the differences between these two patterns of $F^{o}_{1.20}9\text{-}28$ lipoproteins, the schlieren patterns of the serum $d \leq 1.20$ g/ml fractions were examined in earlier frames in which the LDL ($F^{o}_{1.20}28\text{-}56$), $F^{o}_{1.20}9\text{-}28$ lipoproteins, and HDL ($F^{o}_{1.20}0\text{-}9$) were similtaneously present. Figure 31b compares the 14 minute frame from baboons 1664 and 1478. In this frame, the lipoprotein pattern of baboon 1478 shows a distinct peak of flotation rate $F^{o}_{1.20}24$ within the $F^{o}_{1.20}9\text{-}28$ interval which is not present in the pattern from baboon 1664.

Figure 31c compares the analytic ultracentrifugation schlieren patterns of the serum $d \leq 1.063$ g/ml fractions of the same two animals. The pattern of baboon 1664 shows a distinct LDL peak with a flotation rate of $S^{o}_{f}10.0$ and an

Figure 31.

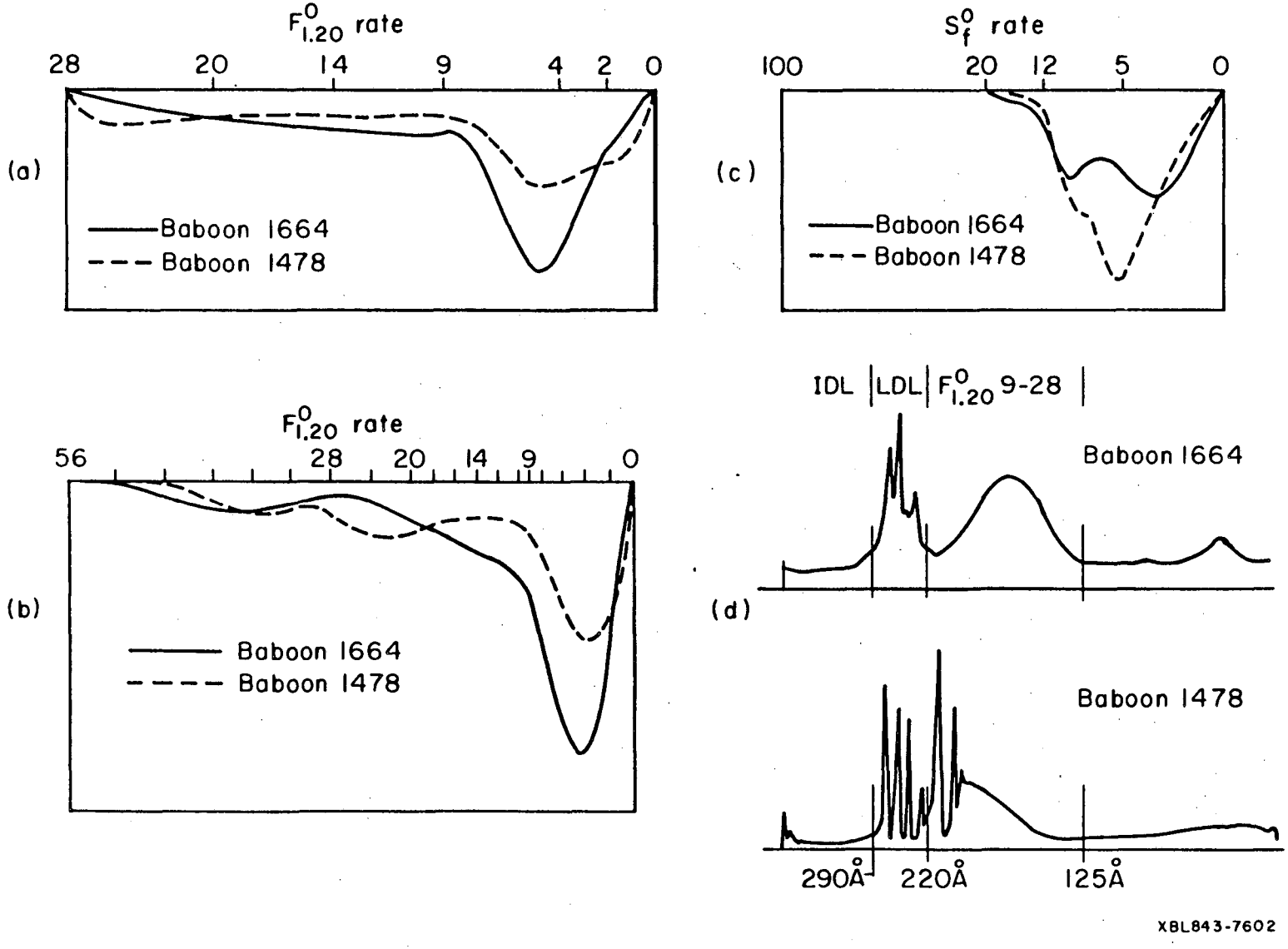
Comparison of the $F^{o}_{1.20}9\text{-}28$ lipoprotein profiles of two baboons (baboon 1664,——; baboon 1478,----) with high levels of $F^{o}_{1.20}9\text{-}28$ lipoproteins while consuming the coconut oil plus cholesterol diet. Baboon 1664 had an $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration of 245 mg/dl, and baboon 1478 had a concentration of 236 mg/dl.

(a). Comparison of the analytic ultracentrifugal schlieren patterns (30 minute frame) of the $F^{o}_{1.20}9\text{-}28$ lipoproteins within the serum $d \leq 1.20$ g/ml fraction.

(b). Comparison of the analytic ultracentrifugal schlieren patterns (14 minute frame) of the $d \leq 1.20$ g/ml fraction showing LDL ($F^{o}_{1.20}28\text{-}56$), $F^{o}_{1.20}9\text{-}28$ lipoproteins and HDL ($F^{o}_{1.20}0\text{-}9$).

(c). Comparison of the analytic ultracentrifugal schlieren patterns (30 minute frame) of the serum $d \leq 1.063$ g/ml fraction.

(d). Comparison of the gradient gel electrophoretic patterns (2-16% gradient gel) of the serum $d \leq 1.063$ g/ml fraction. Baboon 1664 is at top.


(a)
$F^0_{1.20}$ rate
28 20 14 9 4 2 0
Baboon 1664
Baboon 1478
(b)
$F^0_{1.20}$ rate
56 28 20 14 9 0
Baboon 1664
Baboon 1478
(c)
S^0_f rate
100 20 12 5 0
Baboon 1664
Baboon 1478
(d)
IDL | LDL | $F^0_{1.20}$ 9-28
Baboon 1664
Baboon 1478
290Å
220Å
125Å
XBL843-7602

$F^{o}_{1.20}$9-28 lipoprotein peak (contained within the S^{o}_{f}0-5 interval) with a flotation rate of S^{o}_{f}2.3. In contrast, the pattern of baboon 1478 shows a broad peak at S^{o}_{f}5.0 within the S^{o}_{f}0-7 flotation interval. This peak overlaps the LDL peak which falls in the S^{o}_{f}5-12 interval and has a flotation rate of S^{o}_{f}8.7.

Major differences between the particle size distribution of the $F^{o}_{1.20}$9-28 lipoproteins of the two animals appeared when they were compared by gradient gel electrophoresis (2-16% gels) (Figure 31d). The typical pattern (baboon 1664) showed a broad symmetrical distribution of material in the 125-220A particle size range characteristic of $F^{o}_{1.20}$9-28 lipoproteins, while the other pattern (baboon 1478) showed a skewing of the distribution to larger particle sizes and two sharp peaks which correspond to particle sizes 209A and 197A. These peaks were seen only in the gradient gel electrophoresis profiles of these two unusual animals (of twelve examined), both of which had $F^{o}_{1.20}$9-28 lipoprotein concentrations greater than 235 mg/dl. They were not observed in the gradient gel electrophoretic patterns of other baboons with comparable $F^{o}_{1.20}$9-28 lipoprotein concentrations.

Chapter VII: Effects of Saturation of Dietary Fat and Amount of Dietary Cholesterol on the Lipoproteins of Adult Baboons - Experiment III

1. Experimental Design

Ninety-six baboons were divided into four groups of 24 animals each. Each group was fed a diet high in either saturated fat (P/S ratio = 0.26) or polyunsaturated fat (P/S ratio = 2.1) and either high (0.9 mg/Kcal) or low (0.013 mg/Kcal) in cholesterol. Plasma samples obtained from 36 of these baboons, after consuming their assigned diets for six years, were utilized for all studies described in this Chapter. More information on these baboons is provided in Chapter II, Section 1.5.

2. Concentrations of HDL and $F^{o}_{1.20}9\text{-}28$ Lipoproteins within the Serum d<1.20 g/ml Fraction

Table 25 lists the mean ± SD concentrations of total HDL, HDL subpopulations and $F^{o}_{1.20}9\text{-}28$ lipoproteins and HDL peak flotation ($F^{o}_{1.20}$) rate for the four dietary groups examined. Since the four groups were not balanced by sex or by sire, statistical comparisons were not made. However, there are some general trends to note. Among all four groups, the total HDL concentrations were lower than observed in either Experiment I or Experiment II. Baboons on the two diets high in cholesterol tended to exhibit higher mean concentrations of total HDL. Associated with these higher mean HDL concentrations were higher mean concentrations of HDL-I. Other HDL subpopulation concentrations were unaffected by diet. Mean HDL peak $F^{o}_{1.20}$ rates were higher, in general, for baboons on the two saturated fat diets. Mean $F^{o}_{1.20}9\text{-}28$ lipoprotein levels were very low or negligible on all diets.

Table 26 lists the mean ± SD concentrations of total HDL, HDL subpopulations and $F^{o}_{1.20}9\text{-}28$ lipoproteins and the HDL peak $F^{o}_{1.20}$ rates for each dietary group for both males and females. Because of the small number of subjects involved and because there were sire differences between groups, statistical comparisons were not performed. However, there are some trends to note. Among baboons on the high cholesterol-polyunsaturated fat diet, males tended to have higher total HDL concentrations, higher HDL-I

Table 25. Mean ± SD serum concentrations (mg/dl) of HDL and $F^{o}_{1.20}9\text{-}28$ lipoproteins and peak $F^{o}_{1.20}$ rate (svedbergs) within the serum d≤1.20 g/ml fraction for all progeny studied in Experiment III.

Lipoprotein Species	Diet			
	LC-PF (n=12)	LC-SF (n=8)	HC-SF (n=8)	HC-PF (n=8)
HDL-I	179±63	209±38	238±61	240±121
HDL-II	128±17	131±22	120±14	121±29
HDL-III-V	47±16	46±10	47±13	47±15
$F^{o}_{1.20}9\text{-}28$	13	22	11±18	16±25
Peak $F^{o}_{1.20}$ Rate	3.92±.72	4.45±.71	4.67±.47	4.24±1.33

LC-PF: Low Cholesterol-Polyunsaturated Fat Diet
LC-SF: Low Cholesterol-Saturated Fat Diet
HC-SF: High Cholesterol-Saturated Fat Diet
HC-PF: High Cholesterol-Polyunsaturated Fat Diet

Table 26. Mean ± SD concentrations (mg/dl) of HDL and $F^{o}_{1.20}$9-28 lipoproteins and peak $F^{o}_{1.20}$ rate (svedbergs) within the serum d≤1.20 g/ml fraction for male and female baboons in Experiment III.

	Diet			
	LC-PF	LC-SF	HC-SF	HC-PF
Number of Males	8	5	3	3
Number of Females	4	3	5	5
HDL				
Males	348±54	399±58	419±65	467±53
Females	364±47	363±27	397±55	374±86
HDL-I				
Males	176±70	232±24	250±81	339±75
Females	184±54	172±23	231±56	181±105
HDL-II				
Males	123±19	125±27	123±16	95±32
Females	136±7	140±5	119±14	137±14
HDL-III-V				
Males	48±15	43±10	47±14	33±8
Females	43±18	50±11	48±13	56±10
$F^{o}_{1.20}$9-28				
Males	1±2	2±3	8±13	20±22
Females	2±3	2±1	13±22	13±29
Peak $F^{o}_{1.20}$ Rate				
Males	4.02±.85	4.89±.31	4.81±.46	5.34±.30
Females	3.73±.40	3.70±.50	4.58±.51	3.59±1.27

concentrations, faster HDL peak $F^{o}_{1.20}$ rates, but lower HDL-II concentrations.

3. LDL, IDL and VLDL Concentrations within the Serum d<1.063 g/ml Fraction

Table 27 shows the mean ± SD concentrations of LDL, IDL and VLDL and the peak LDL flotation (S^{o}_{f}) rate for the four dietary groups studied. Mean LDL concentrations were approximately 70% higher on each of the high cholesterol diets relative to each of the low cholesterol diets (135 ± 38 mg/dl on high cholesterol-saturated fat vs 81 ± 13 mg/dl on low cholesterol-saturated fat; 108 ± 40 mg/dl on high cholesterol-polyunsaturated fat vs 61 ± 20 mg/dl on low cholesterol-polyunsaturated fat). Mean LDL concentrations were also approximately 27% higher on each of the saturated fat diets relative to each of the polyunsaturated fat diets (high cholesterol-saturated fat vs high cholesterol-polyunsaturated fat; low cholesterol-saturated fat vs low cholesterol-polyunsaturated fat). Mean IDL concentrations were <25 mg/dl on all diets, but tended to be higher on the two high cholesterol diets. Mean VLDL levels were <10 mg/dl on all diets. The mean peak S^{o}_{f} rates were higher on the two saturated fat diets relative to the two polyunsaturated fat diets.

Table 28 lists the mean ± SD concentrations of LDL, IDL and VLDL and the peak S^{o}_{f} rate for the baboons on the four diets grouped by sex. The mean LDL concentrations for females were slightly higher on all diets (approximately 10% higher than males on the two high cholesterol diets; 20% higher on the low cholesterol-saturated fat diet and 60%

Table 27. Mean ± SD serum concentrations (mg/dl) of LDL, IDL and VLDL and peak S^{o}_{f} rate (svedbergs) within the serum d≤1.063 g/ml fraction from all the baboons in Experiment III.

Lipoprotein Species	Diets			
	LC-PF	LC-SF	HC-SF	HC-PF
LDL	61±20	81±13	135±38	108±40
IDL	9±8	8±6	15±10	23±13
VLDL	3±3	7±8	5±3	7±4
Peak S^{o}_{f} Rate	7.96±.50	8.62±.52	8.56±.23	7.94±1.10

LC-PF: Low Cholesterol-Polyunsaturated Fat Diet
LC-SF: Low Cholesterol-Saturated Fat Diet
HC-SF: High Cholesterol-Saturated Fat Diet
HC-PF: High Cholesterol-Polyunsaturated Fat Diet

Table 28. Mean ± SD serum concentrations (mg/dl) of LDL, IDL and VLDL and peak S^{o}_{f} rate for male and female baboons in Experiment III.

	Diets			
	LC-PF	LC-SF	HC-SF	HC-PF
Number of Males	8	5	3	3
Number of Females	4	3	5	5
LDL				
Males	50±9	75±12	126±43	96±10
Females	81±22	90±12	140±39	115±51
IDL				
Males	8±5	4±3	10±7	23±5
Females	11±13	14±6	18±11	23±17
VLDL				
Males	3±1	4±3	3±2	8±6
Females	4±6	11±12	6±4	7±4
Peak S^{o}_{f} Rate				
Males	7.97±.60	8.45±.54	8.56±.12	8.97±.42
Females	7.94±.23	8.90±.41	8.56±.29	7.33±.88

higher on the low cholesterol-polyunsaturated fat diet).

Chapter VIII: Discussion

1. Characterization of Baboon (Papio cynocephalus) Lipoproteins

1.1. High Density Lipoproteins

High density lipoproteins (HDL) in the baboon are a major transport vehicle for cholesterol, carrying as much as 50% of the total serum cholesterol (43). Serum concentrations of HDL in baboons, reported in this dissertation, range between 300 and 700 mg/dl, regardless of diet or genetic background. These concentrations are high relative to those normally observed in humans, but are similar to levels encountered in other non-human primate species (8,65,66). Baboon HDL are confined mainly to the ultracentrifugal $F^{o}_{1.20}0\text{-}9$ flotation interval, which also defines human HDL (71,86).

Gradient gel electrophoresis of baboon HDL indicates considerable polydispersity within this lipoprotein class; 5 discrete peaks (or subpopulations) are observed in densitometric scans of these gels. The particle size of each lipoprotein subpopulation appears to be similar for all baboons and is unaffected by diet or genetic background. This fact is demonstrated when the relative migration distance (R_f value) of each electrophoretic peak in the HDL a large number of baboons is tabulated and plotted in a histogram (Figure 2,n=46; Figure 21,n=30; Figure 30,n=45). In

each instance, the resulting distribution reveals five discrete modes between particle sizes 72-125A. Size subintervals containing these modes were used to define five baboon HDL subpopulations: HDL-I through HDL-V from largest to smallest mean diameter. The size ranges of the baboon HDL subpopulations defined in this way were not affected either by diet (Figures 21 and 30) or by genetic background (Figure 2). This lends support to the concept that the discrete peaks observed within baboon HDL represent distinct HDL subpopulations. Five discrete HDL subpopulations have been observed by this method in plasma HDL of humans, whereas dog HDL (87) and rat HDL (88) exhibit two or three subclasses. The data in this dissertation are the first to describe the HDL of an animal species, other than humans, that exhibits such extensive and consistent polydispersity within the HDL class.

Baboon HDL subpopulations were separable by density gradient ultracentrifugation, making compositional analysis possible (Table 6). Clear trends of increasing percent protein and decreasing percent phospholipid, percent unesterified cholesterol and particle size were observed with increasing density. Compositional data on baboon HDL subpopulations have not been previously reported. Reported composition values for whole HDL (18,37) and "HDL_2" and "HDL_3" subclasses (43) are consistent with the data in this dissertation. Human HDL subpopulations (also five modes within a particle size range of 72-125A) are very similar in size,

density and chemical composition to their baboon counterparts. The similarities in HDL structure between humans and baboons imply comparable structural roles for the HDL apolipoproteins of the two species, though the metabolic properties of baboon apolipoproteins (e.g. receptor binding activity, enzymatic cofactor activity) are not well documented.

The apolipoprotein content of baboon HDL subpopulations was examined by sodium dodecyl sulfate-polyacrilamide gel electrophoresis and indicated that apoA-I (2.8×10^4 daltons) was the major apolipoprotein in all HDL subpopulations. All subpopulations also possessed small molecular weight proteins (1.8×10^4, 1.4×10^4, 1.3×10^4 and 1.1×10^4 daltons) which are probably apoA-II and forms of apoC. Densitometric scanning of bands on the sodium dodecyl sulfate-polyacrilamide electrophoretic gels indicated that the relative amounts of these apolipoproteins may differ among the HDL subpopulations. However, the quantification of closely spaced individual bands by densitometric scanning was not reliable because of a lack of resolution. ApoE was not detected in any baboon HDL subpopulation. Data on Baboon apolipoproteins have been previously reported by several investigators (8,35-41). Bojanovski et al (18) found that baboon whole HDL possesses a collection of apolipoproteins identical to that presented in this dissertation, except that all their proteins were assigned somewhat higher molecular weights. They also demonstrated that baboon

apolipoproteins cross-react with antibodies raised against human apoA-I, apoA-II, apoB, apoC, apoD and apoE, but no mention is made whether this cross-reactivity is quantitative. Baboon apoA-I and apoA-II have been sequenced (35) and show considerable homology to the human proteins. Of note is the observation that baboon apoA-II lacks the cysteine residue at amino acid position 6 found only in the human (89), chimpanzee (90) and pink salmon (91) apolipoprotein. Consequently, baboon apoA-II appears as a monomer in plasma, not the dimer usually seen in human plasma.

Since baboon apoA-II does not have a cysteine residue, apo(E-A-II) complexes are not formed. These mixed complexes are found in some large HDL species of humans (92). Innerarity et al (93) have shown that the apo(E-A-II) complex does not bind to cellular receptors and, therefore, is not as readily removed from plasma as uncomplexed apoE. This could explain why apoE is observed in small, but measurable amounts in human HDL, but not detected in baboon HDL. That is, apoE in baboon HDL would be receptor active and removed from plasma rapidly. In contrast, apoE in human HDL may be in the receptor inactive apo(E-A-II) complex and would remain in circulation for a relatively longer time.

Using the measured biophysical properties of baboon HDL subpopulations, it was possible to calculate their flotation properties and then, from the analytic ultracentrifugal schlieren patterns, estimate the concentrations of individual HDL subpopulations. The results indicate that HDL-I

is, by far, the major baboon HDL subpopulation and that differences in HDL-I concentrations (and, to a lesser extent, HDL-II) account for most of the variations in HDL concentrations observed in all studies. Among humans, HDL_3 (corresponding to baboon HDL-III through HDL-V) is often predominant among males (44), while females may possess roughly equal concentrations of HDL_3, HDL_{2a} (corresponding to baboon HDL-II) and HDL_{2b} (corresponding to baboon HDL-I). In humans, though, HDL_{2b} (the HDL in humans of largest particle size) is often most variable among individuals, just as HDL-I (the HDL in baboons of largest particle size) shows the most variation among baboons.

Although baboon and human HDL exhibit somewhat similar properties, such as particle size, composition and the presence of distinct subpopulations, the plasma levels of HDL of humans and baboons are quite different. As reported in this dissertation, baboon mean HDL concentrations are often between 400 and 500 mg/dl, while human HDL concentrations are generally between 200 and 400 mg/dl (44,86). In addition, while approximately 60% of baboon HDL lipoprotein material is found within the HDL-I subpopulation (particle size 100-125A, hydrated density 1.063-1.120 g/ml), the distribution of human HDL mass is shifted to smaller size particles (particle size 82-100A, hydrated density 1.110-1.150 g/ml).

1.2. $F^{o}_{1.20}9\text{-}28$ Lipoproteins

$F^{o}_{1.20}9\text{-}28$ lipoproteins are a heterogeneous class of lipoproteins of size 125-220A, of hydrated density 1.028-1.080 g/ml and possess the same apolipoproteins observed in baboon HDL with the addition of apoE (3-11% of total protein in individual subfractions). Most of their properties are similar to the HDL_c species ("cholesterol-induced HDL") reported in cholesterol-fed dogs (16), swine (12) and rats (59). Based on the studies reported in this dissertation, $F^{o}_{1.20}9\text{-}28$ lipoproteins are induced in baboons by dietary cholesterol, but only on diets which also include a high amount of saturated fat and, then, only in those baboons that exhibit a hypercholesterolemic response to such a diet. The dietary and genetic control of the $F^{o}_{1.20}9\text{-}28$ lipoproteins will be discussed in a later section.

The $F^{o}_{1.20}9\text{-}28$ lipoprotein class exhibits considerable heterogeneity; the subfractions of lower hydrated density are larger in particle size, possess a lower percent protein and greater percent cholesteryl ester and are considerably richer in apoE. The extent of heterogeneity within the $F^{o}_{1.20}9\text{-}28$ lipoprotein class of an individual baboon depends strongly upon the total $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration of that animal. That is, when the animal possesses low concentrations of $F^{o}_{1.20}9\text{-}28$ lipoproteins, only the small, dense subspecies within the $F^{o}_{1.20}9\text{-}28$ lipoprotein spectrum are present. At higher concentrations, both small (dense) and large (less dense) subspecies are present. The

relationship between particle size distribution and serum concentration may reflect the direction of metabolism of these lipoproteins. This concept is supported by the studies in which dietary manipulations resulted in changes in $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations (Experiment I, Chapter V and Experiment II, Chapter VI). When the baboons' diets were changed to increase the level of serum $F^{o}_{1.20}9\text{-}28$ lipoproteins, the smaller species were always present in the serum before the larger species appeared. Similarly, when diets were adjusted in order to decrease the concentration of $F^{o}_{1.20}9\text{-}28$ lipoproteins, the larger species were reduced while the smaller species were still apparent. Additional in vivo studies are necessary, of course, to establish whether such a relationship between the small and large species within the $F^{o}_{1.20}9\text{-}28$ spectrum is indeed the case.

One component contributing to the heterogeneity of the $F^{o}_{1.20}9\text{-}28$ lipoprotein spectrum is apoE. In the present studies, apoE accounted for 11% of the protein in the larger $F^{o}_{1.20}9\text{-}28$ lipoprotein species, and approximately 3% of the protein of the smaller species. Consequently, it appears that the $F^{o}_{1.20}9\text{-}28$ lipoproteins consist of at least two subpopulations: one small, dense and with a low content of apoE, and one large, less dense and with a higher content of apoE. It is most likely the presence of apoE within the larger size $F^{o}_{1.20}9\text{-}28$ lipoprotein subpopulation which accounts for the observation that this subpopulation is precipitable by heparin-manganese chloride. Therefore,

heparin-manganese precipitation (94) or heparin-sepharose affinity chromatography (95) could be a useful method of separating these two subpopulations for further characterization.

Additional evidence for the heterogeneity of the $F^{o}_{1.20}9\text{-}28$ lipoproteins is the observation of two discrete peaks (209A and 197A) within the $F^{o}_{1.20}9\text{-}28$ lipoprotein particle size range in gradient gel electrophoretic patterns of some baboons with high levels of $F^{o}_{1.20}9\text{-}28$ lipoproteins. These baboons also exhibited markedly elevated levels of lipoproteins within the $F^{o}_{1.20}20\text{-}28$ flotation range (peak of $F^{o}_{1.20}24$). When analyzed in the $d \leq 1.063$ g/ml serum fraction, these lipoproteins appeared as a broad peak of flotation rate $S^{o}_{f}5$. Isolation and further characterization of these discrete species is the subject of future studies, as is selective breeding for this trait among baboon families.

Mahley et al (16) have indicated that HDL_c in dogs is also heterogeneous with regard to apoE content. They have described a subpopulation that contains exclusively apoE, with hydrated density of $d \leq 1.019$ g/ml and of size ranging up to 400A. Comparable species with these properties have not been observed in either baboons or humans.

Human HDL species that contain some apoE (9,96-100) are larger (100-150A) than typical human HDL, can be increased in concentration by high cholesterol diets and have been designated human "HDL-with-E". Although baboon $F^{o}_{1.20}9\text{-}28$ lipoproteins are larger than human HDL-with-E, the

possibility of comparable metabolic origins and fates of these two lipoprotein species should be considered.

The origin of $F^{o}_{1.20}9\text{-}28$ lipoproteins is not yet known. Two investigators have proposed that the metabolic precursors of HDL_c are present in dog lymph (101-103) and dog plasma (87) and that their conversion might occur during *in vitro* incubations of isolated lymph or plasma, respectively, in the presence of active lecithin:cholesterol acyltransferase and added cholesterol. While the products of these incubations were not well characterized they did differ from the HDL_c normally found in dog plasma in both hydrated density and chemical composition. When similar incubations were performed on the plasma from fasted baboons, there were no changes observed in either the concentration or particle size distribution of $F^{o}_{1.20}9\text{-}28$ lipoproteins (88). Clearly, additional *in vitro* and *in vivo* studies are required to elucidate the metabolism of the $F^{o}_{1.20}9\text{-}28$ lipoproteins.

One possible mechanism that might apply to the origin of the $F^{o}_{1.20}9\text{-}28$ lipoproteins has been proposed (104,105) to explain a metabolic relationship between small HDL particles (HDL_3) and large HDL particles (HDL_2). This model proposes that HDL_2 particles are derived from HDL_3 particles by acquiring phospholipid, cholesterol and apolipoproteins from partially delipidated VLDL and chylomicrons and are then remodeled by the action of lecithin:cholesterol acyltransferase. Specifically, the triglyceride molecules that

make up most of the non-polar lipid core of VLDL and chylomicrons would be hydrolyzed by lipoprotein lipase (in vascular beds) and possibly hepatic lipase (in the liver), producing a core-deficient (or surface-rich) remnant particle. Residual, or redundant, surface components (phospholipid, cholesterol and apolipoproteins) might pinch off from this remnant particle, and remain in the plasma as vesicular or disc-like structures (106,107). These structures may combine with circulating HDL_3 particles and provide additional substrate for lecithin:cholesterol acyltransferase. Lecithin:cholesterol acyltransferase would mediate the conversion of phospholipid and cholesterol (surface components) into cholesteryl ester (a core component) and lysolecithin (which would be transferred to albumin). The altered HDL_3 particle would be larger because it has acquired both surface (cholesterol, phospholipid and apolipoproteins) and core (cholesteryl ester) material and, hence, would resemble an HDL_2 particle. In like manner then, a mechanism to explain the origin of $F^{o}_{1.20}9\text{-}28$ lipoproteins might involve, first, an uptake of phospholipid-cholesterol-apolipoprotein material (from lipolysis of VLDL and chylomicrons) by circulating HDL-I particles and, secondly, a remodeling of this altered HDL-I via lecithin:cholesterol acyltransferase. Such a mechanism seems feasible because baboon HDL are comprised predominantly of HDL-I (the largest HDL subpopulation) and because the saturated fat and cholesterol diets which are found to

induce elevations of $F^{o}_{1.20}9\text{-}28$ lipoprotein material also result in increased production of chylomicrons and VLDL.

A second mechanism for the origin of $F^{o}_{1.20}9\text{-}28$ lipoproteins might involve conversion of precursor species, such as apoE-rich phospholipid and cholesterol discoidal complexes secreted directly by the liver. Interaction of these complexes with lecithin:cholesterol acyltransferase would convert surface components (cholesterol and phospholipid) to the core component, cholesteryl ester, and transform the discs into spherical structures. ApoE-containing phospholipid and cholesterol disc-like structures have been observed in extracellular media following perfusion of rat (108,109) and guinea pig (110) livers and also appear in the plasma of individuals with lecithin:cholesterol acyltransferase-deficiency (111-114). Following incubation in the presence of active lecithin:cholesterol acyltransferase, these disc-like structures are converted to spheres, with properties approximating circulating HDL (115).

Clearly, elucidation of the origin and degradation of $F^{o}_{1.20}9\text{-}28$ lipoproteins cannot be determined by studying the plasma lipoproteins of fasted animals, but requires careful metabolic studies.

1.3. LDL, IDL and VLDL

Concentrations of lipoprotein classes within the serum d≤1.063g/ml fraction were measured by analytic ultracentrifugation. LDL was measured within the $S^{o}_{f}5$-12 flotation interval, IDL within the $S^{o}_{f}12$-20 interval and VLDL within the $S^{o}_{f}20$-100 interval. Human LDL is usually measured within the $S^{o}_{f}0$-12 flotation interval. Baboon LDL, in fact, often includes a small amount of material with flotation rates below $S^{o}_{f}5$. This constitutes but a small fraction of the total LDL material and is often masked by elevated concentrations of $F^{o}_{1.20}9$-28 lipoprotein material which floats within the $S^{o}_{f}0$-5 flotation interval. For consistency in the present study, LDL was always measured within the $S^{o}_{f}5$-12 flotation interval, regardless of the presence of $F^{o}_{1.20}9$-28 lipoprotein material.

LDL is, by far, the predominant lipoprotein species within the $S^{o}_{f}5$-100 flotation rate range. concentrations of LDL in the plasma of baboons in this study ranged between 80 and 500 mg/dl and demonstrated some modulation by diet and genetic background. Baboon LDL always appears in analytic ultracentrifugal schlieren patterns as a single peak, generally with a peak flotation rate between $S^{o}_{f}7$ and $S^{o}_{f}10$. The LDL peak flotation rate also seems to be strongly dependent upon dietary and genetic factors.

LDL examined by gradient gel electrophoresis demonstrates a polydisperse character; LDL of individual baboons in this study exhibited between one and five discrete

lipoprotein peaks within the 220-290A particle size range. To determine if the discrete LDL peaks observed by gradient gel electrophoresis represent distinct LDL subpopulations, the relative migration distance (R_f value) of each electrophoretic peak in the LDL of all baboons in Experiment II was tabulated and plotted as a frequency histogram (Figure 28,n=72). The resulting distribution indicates seven distinct modes between particle sizes 220-290A. Size subintervals containing these modes were used to define seven baboon LDL subpopulations, designated A through G from largest to smallest diameter.

Human LDL, which is polydisperse when examined by gradient gel electrophoresis, also exhibits seven distinct particle size subpopulations when the frequency of occurrence of discrete peaks of specific particle size is plotted versus the particle size (48). While the overall particle size range of LDL for humans (approximately 220-278A) and baboons (220-290A) is quite similar, the magnitude of the particle size ranges of individual subpopulations is quite different. Whereas the particle size subintervals containing baboon LDL subpopulations are each approximately 10A, the subintervals for human LDL subpopulations range between 4 and 15A.

Density gradient ultracentrifugation does not resolve individual LDL subpopulations. As shown in Figures 9 and 15, individual density subfractions were often polydisperse, though there was a trend of decreasing particle size with

increasing hydrated density. In the serum of all baboons studied by density gradient ultracentrifugation, two distinct LDL bands are apparent: one at the top of the tube ($d \leq 1.025$ g/ml) and a second band near the middle of the tube (d 1.028-1.040 g/ml). ApoB is the major apolipoprotein in both bands and these two fractions exhibited similar chemical compositions (Table 11). The compositions of LDL subfractions reported in this dissertation are comparable to those of human LDL subfractions of similar hydrated density, as reported by Shen et al (72).

The significance and origins of LDL subpopulations are still unknown. LDL subfractions are reported to exhibit differing reactivities to LDL monoclonal antibodies (116). Such differences may reflect different degrees of masking of apoB antigenic sites by phospholipid, cholesterol or, perhaps, lower molecular weight apolipoprotein molecules on the particle surface. At present, it is not known whether the presence of LDL subpopulations is due to differences in lipid composition and/or content between LDL particles or to the existence of several specific conformations of apoB (the major, if not exclusive, apolipoprotein in LDL) which may, in turn, be modulated by the lipid molecules within the LDL particle.

1.4. Interrelationships among Concentrations of Lipoprotein Classes

Significant positive correlations were observed among VLDL, IDL and LDL concentrations and LDL peak S^{o}_{f} rates among all progeny consuming the LARD+CS diet in Experiment I (Table 10). Krauss et al (49), working with human subjects, observed significant positive correlations among concentrations of VLDL, IDL and specific subfractions of LDL; no negative correlations were noted.

Among baboon HDL subpopulations, HDL-III through HDL-V concentrations were negatively correlated with HDL-I concentrations and positively correlated with HDL-II concentrations (Table 7). HDL-I concentrations and HDL-II concentrations were unrelated among all 46 progeny used in Experiment I, but HDL-I and HDL-II concentrations were positively correlated among the 36 progeny of the three hypercholesterolemic sires. Correlations among HDL subpopulations of humans, consuming an ad lib diet, are somewhat different (44). Among human HDL subpopulations, HDL_3 (corresponding to baboon HDL-III through HDL-V) concentrations are negatively correlated with HDL_{2b} (corresponding to baboon HDL-I) concentrations, but exhibit a non-significant negative correlation with HDL_{2a} (corresponding to baboon HDL-II) concentrations. In addition, human HDL_{2b} (corresponding to baboon HDL-I) concentrations and HDL_{2a} (corresponding to baboon HDL-II) concentrations are positively correlated. While baboons and humans demonstrate

different statistical correlations among corresponding HDL subpopulations, this does not necessarily indicate that the metabolic relationships between HDL subpopulations are radically different in baboons vs humans. Baboons possess considerably higher concentrations of large HDL (HDL-I in the baboon, corresponding to HDL_{2b} in the human) and considerably lower concentrations of small HDL (HDL-III through HDL-V in the baboon, HDL_3 in the human) than humans. Comparisons of correlation coefficients calculated over different concentration ranges are difficult to interpret because most relationships between lipoprotein subpopulation concentrations are curvilinear (44).

Linear correlations calculated between concentrations of baboon HDL subpopulations and LDL, IDL and VLDL concentrations presented in this dissertation (Table 10) are quite similar to those correlation coefficients calculated by Anderson (44) for the corresponding human lipoprotein concentrations. In general, concentrations of lipoproteins in the serum $d \leq 1.063$ g/ml fraction were inversely related to HDL-I and HDL-II concentrations, but positively correlated to HDL-III through HDL-V concentrations.

Correlation coefficients among lipoprotein levels, measured in a population of individuals, do not provide direct information regarding the underlying metabolic relationships among lipoproteins. However, differences in such correlation coefficients calculated for two populations may indicate, indirectly, the presence of metabolic differences

between these two populations (e.g. dietary, genetic or hormonal differences). In this regard, the similarity between correlation coefficients for baboons and humans strongly suggests that baboons may indeed serve as an appropriate animal model of human lipoprotein metabolism. This view is reinforced by the characterization data that indicate similarities in biophysical properties (e.g. chemical composition, flotation characteristics, particle size and discrete subpopulation distributions) of the lipoproteins of baboons and humans.

2. Modulation of Baboon Lipoproteins by Dietary Fat and Cholesterol

2.1. High Density Lipoproteins

One result of the experiments reported in this dissertation is that dietary saturated fat increases the serum concentration of HDL in the baboon. This effect was observed in two separate experiments. In one study (Chapter VI), baboons exhibited higher concentrations of HDL (measured as lipoprotein material within the $F^{o}_{1.20}0$-9 flotation rate range) when consuming a diet high in saturated fat (coconut oil) and cholesterol than when the same baboons were consuming a diet high in polyunsaturated fat and cholesterol. In a separate study (Chapter V), baboons exhibited higher HDL levels when consuming a diet high in saturated fat (lard), but low in cholesterol, than those observed when consuming a CHOW diet. An additional finding of the present study is that cholesterol exhibits an HDL-lowering effect when it is added to diets high in saturated fat (lard), (Chapter V). Concommitant with this decrease in HDL, an elevation of $F^{o}_{1.20}9$-28 lipoproteins is observed.

The positive effect of dietary saturated fat (relative to polyunsaturated fat) on HDL concentrations has been reported by several investigators (60,117-121) in humans and other animal species. This effect has been attributed to a higher intestinal apoA-I synthesis of a saturated fat diet (122). The HDL-lowering effect of dietary cholesterol has

been reported by Mahley et al (9) in humans and in other species. In addition, many species exhibit a concommitant increase in $F^{o}_{1.20}9$-28-like lipoproteins (HDL_C in some species, HDL-with-E in humans) (9,12,16,59,99), while others do not (11).

In the baboon model, the concentrations of individual HDL subpopulations respond differently to the type of dietary fat and the amount of dietary cholesterol. HDL-I levels are increased by dietary saturated fat both without cholesterol (lard diet relative to the CHOW diet) and with cholesterol (coconut oil diet relative to the corn oil diet). HDL-I levels, though, are decreased by the addition of dietary cholesterol to the lard diet. HDL-II concentrations are unchanged by differences in dietary fat or cholesterol. HDL-III through HDL-V concentrations are increased by the addition of cholesterol (to the lard diet) but are unaffected by type or amount of dietary fat. In the only dietary studies performed on humans which measured concentrations of HDL subpopulations (46,47) saturated fat was found to increase HDL_{2b} levels relative to those on a polyunsaturated fat diet. This observation is analogous to the results reported in this dissertation for baboon HDL-I concentrations.

Examination of HDL particle size distributions by gradient gel electrophoresis demonstrated that the amount of dietary cholesterol and the type or amount of dietary fat has little effect on the size intervals defining the HDL

subpopulations (compare Figures 2, 21 and 30). Under some circumstances, though, some subpopulations - especially HDL-V - were difficult to detect in every animal. There are no data in other species describing size intervals of HDL subpopulations as a function of diet. It should be noted, though, that the initial observations defining size intervals of HDL subpopulations in humans were made on a population on an ad lib diet.

The data in this dissertation support the concept that modualtion of HDL levels by diet results in different relative amounts of individual HDL subpopulations, rather than specific changes in the biophysical properties of the HDL subpopulations.

2.2. $F^{o}_{1.20}$9-28 Lipoproteins

$F^{o}_{1.20}$9-28 lipoproteins are elevated when baboons are placed on a diet containing high amounts of cholesterol and saturated fat (lard or coconut oil) and are essentially absent on a diet high in cholesterol and polyunsaturated fat or a diet low in fat and cholesterol (CHOW). Consumption of a diet high in saturated fat but with little cholesterol produces modest, but statistically significant, increases in $F^{o}_{1.20}$9-28 lipoproteins relative to a CHOW diet. Therefore, it appears that both saturated fat and cholesterol are required to induce maximal levels of these lipoprotein species.

Lipoproteins with properties similar to those of $F^{o}_{1.20}9\text{-}28$ lipoproteins have been observed in the plasma of dogs, swine and rats (9,12,16,59) when these animals are fed a diet high in cholesterol and saturated fat. This response has been attributed primarily to dietary cholesterol, and hence these lipoproteins are designated HDL_c (cholesterol-induced HDL). However, the experiments in this dissertation show that while $F^{o}_{1.20}9\text{-}28$ lipoproteins can be elicited in many baboons by a diet high in cholesterol and saturated fat, the same animals will not exhibit a comparable $F^{o}_{1.20}9\text{-}28$ lipoprotein response when fed the same level of cholesterol with polyunsaturated fat. Since cholesterol absorption in the baboon is not significantly affected by the type of dietary fat (29), the combined results indicate that dietary cholesterol alone is not sufficient to elicit $F^{o}_{1.20}9\text{-}28$ lipoproteins.

As mentioned in the previous section, the effects of the type of dietary fat on lipoprotein metabolism in humans have been studied by several investigators. Among human subjects, Shepherd et al (122) found that a diet high in saturated fat increased synthesis of apoA-I relative to a diet high in polyunsaturated fat, although the fractional catabolic rates of apoA-I were the same on both diets. This finding is consistent with the observation of higher HDL and $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations (the major pools of apoA-I) in the serum of baboons on the saturated fat diet relative to the polyunsaturated fat diet.

2.3. LDL, IDL and VLDL

Dietary saturated fat (in the presence of cholesterol), relative to dietary polyunsaturated fat, increases LDL concentrations in female baboons but not in male baboons. Among all human subjects, saturated fat tends, on average, to elevate LDL levels (117,119-121,123,124). In baboons, dietary cholesterol, when added to a diet containing lard, increases LDL concentrations. In addition, LDL peak flotation (S^{o}_{f}) rate (measured by analytic ultracentrifugation) and LDL peak particle size (measured by gradient gel electrophoresis) are both increased by the addition of high amounts of lard to a CHOW diet (when increases in LDL concentrations are minimal) and by the addition of cholesterol to the lard diet (when LDL concentrations increase considerably).

IDL and VLDL are virtually absent from baboon serum when the animal is consuming a CHOW diet. IDL are induced to a small extent by saturated fat without cholesterol. Both IDL and VLDL are increased by the addition of cholesterol to a lard diet.

A most interesting observation in the present study, though, is that levels of both IDL and VLDL are increased by polyunsaturated fat relative to those on a saturated fat diet in the presence of cholesterol. This increase in IDL and VLDL could reflect depressed lipolytic activity and decreased transfer of apoE to more dense lipoproteins, with the result that production of $F^{o}_{1.20}$9-28 lipoprotein species

on the polyunsaturated fat diet is prevented.

3. Differential Responses: Sex Differences and Possible Genetic Control

3.1. High Density Lipoproteins

There are no apparent sex differences in total HDL concentrations ($F^{o}_{1.20}0$-9 lipoprotein material by analytic ultracentrifugation) on any diet. In addition, males and females exhibit similar changes in total HDL concentrations in response to changes in diet. There are, however, minor differences in the distribution of mass among HDL subpopulations, with males possessing more HDL-II ($P<0.05$) but less HDL-I ($P<0.1$) on both the COCO+CS and CORN+CS diets. Among adult human subjects on ad lib diets (44), females tend to possess higher HDL_{2b} (corresponding to baboon HDL-I), HDL_{2a} (HDL-II) and total HDL concentrations and, perhaps, slightly lower HDL_3 (HDL-III through HDL-V) levels. Since the species differences (in HDL concentrations) between humans and baboons are far greater than the sex differences encountered in the baboon, this animal species may provide a poor model for the study of the effects of sex-related hormones on lipoprotein metabolism. However, since male and female baboons differ with regard to the concentrations of HDL-I and HDL-II, without differences in total HDL concentrations, the baboon may be a good model for the study of differences in metabolic or physiologic functions among HDL subpopulations.

The results of Experiment I, which involved the progeny of assortatively mated (high total serum cholesterol with high; low total serum cholesterol with low) sires and dams, provide some indication of genetic differences in HDL metabolism in the baboon. Although progeny of hypercholesterolemic sires (families exhibiting $F^{o}_{1.20}9\text{-}28$ lipoproteins) and progeny of hypocholesterolemic sires (families without $F^{o}_{1.20}9\text{-}28$ lipoproteins) possess identical total HDL concentrations, progeny in families with $F^{o}_{1.20}9\text{-}28$ lipoproteins have significantly greater amounts of HDL-I and significantly lower amounts of HDL-II. Since analytic ultracentrifugation does not provide adequate separation of baboon HDL ($F^{o}_{1.20}0\text{-}9$) from $F^{o}_{1.20}9\text{-}28$ lipoproteins, high concentrations of HDL-I in the serum of the progeny of hypercholesterolemic sires may include contamination by $F^{o}_{1.20}9\text{-}28$ species.

When HDL particle size distributions are determined by gradient gel electrophoresis, there appear to be no sex or family differences in the particle size ranges of HDL subpopulations.

3.2. $F^{o}_{1.20}9\text{-}28$ Lipoproteins

The experiments reported in this dissertation indicate that $F^{o}_{1.20}9\text{-}28$ lipoprotein concentrations are affected by both sex and genetic background. In Experiment II (in which baboons near or past puberty were sequentially fed the COCO+CS and CORN+CS diets), males consistently exhibited

higher $F^{o}_{1.20}$9-28 lipoprotein concentrations on the COCO+CS diet. (Such a sex difference in $F^{o}_{1.20}$9-28 lipoprotein concentrations was not observed during Experiment I which involved primarily pre-pubertal baboon progeny.) Since males also exhibit a greater elevation in IDL and VLDL concentrations when switched to the CORN+CS diet (Table 24, diet-by-sex interaction), it is interesting to speculate that male baboons possess more apoE than females and that this apolipoprotein is partitioned between IDL and $F^{o}_{1.20}$9-28 lipoproteins. The partitioning of apoE between these two lipoprotein species would be regulated by lipolytic activity and the availability of apoA-I-containing acceptor lipoproteins (possibly HDL-I) for the apoE moiety. A detailed study of the apoE levels in baboons has not been performed; in humans, females possess, generally, at least as much apoE as males (96-98,100).

Experiment I, involving pedigree progeny, demonstrated that the presence of $F^{o}_{1.20}$9-28 lipoproteins in the serum (when baboons are fed a diet high in saturated fat and cholesterol) is a trait which can be bred for or against. The present studies did not measure serum levels of individual apolipoproteins (e.g. apoA-I and apoE) nor serum enzymatic activity (e.g. lecithin:cholesterol acyltransferase activity). Therefore, it is not possible to identify what metabolic differences between baboon families may account for the presence or absence of $F^{o}_{1.20}$9-28 lipoproteins in the sera of these animals.

In addition, experiments were not performed to show whether the progeny of hypocholesterolemic sires (which did not exhibit $F^{o}_{1.20}9\text{-}28$ lipoproteins) could be made to demonstrate elevated levels of $F^{o}_{1.20}9\text{-}28$ lipoproteins if their serum cholesterol levels could be increased by other than dietary means. Therefore, elevation in levels of $F^{o}_{1.20}9\text{-}28$ lipoproteins should be considered one aspect of diet-induced hypercholesterolemia which is observed, to a greater or lesser extent, in the sera of baboons. Among baboons that are hypocholesterolemic, in response to high saturated fat and cholesterol diets, $F^{o}_{1.20}9\text{-}28$ lipoproteins are essentially absent from the serum.

The $F^{o}_{1.20}9\text{-}28$ lipoproteins of almost all baboons on saturated fat and cholesterol diets show similar heterogeneity in particle size (Figure 6) and flotation ($F^{o}_{1.20}$) rate distribution (Figure 5) as a function of total $F^{o}_{1.20}9\text{-}28$ lipoprotein concentration. There are exceptions, however, as illustrated by baboon 1664 in Figure 31. This animal (while consuming the COCO+CS diet) exhibits elevated levels of faster floating species (peak flotation rates of $F^{o}_{1.20}24$ and $S^{o}_{f}5$) which appear as two discrete species by gradient gel electrophoresis (particle sizes of 209A and 197A). In studies not reported in this dissertation, these animals were found to exhibit unusually high levels of apoE (125). The physical-chemical properties of these lipoprotein are yet to be established. It is possible that these species are similar to a unique HDL_c subspecies, which

contain only apoE and appear as a single, discrete peak on gradient gel electrophoresis patterns (though of somewhat larger particle size) in the serum of hypercholesterolemic dogs as reported by Gordon et al (87).

3.3. LDL, IDL and VLDL

The data from Experiment II (in which baboons were sequentially fed the COCO+CS and CORN+CS diets) show that female baboons have significantly higher LDL concentrations than males, on both the COCO+CS and CORN+CS diets. In addition, while female LDL levels were somewhat higher on the COCO+CS diet, male LDL levels were indistinguishable on the two diets. These results are quite interesting since human females generally exhibit lower LDL levels that males (86). No other reports of sex differences in lipoprotein concentrations in baboons are available. One point to consider is that of the 24 baboons selected for Experiment II, only 12 were examined by analytic ultracentrifugation for more than one diet period; data from these 12 were used in the final analysis. The possibility that the choice of these 12 from the original 24 was biased in some way should not be discounted. An indication of possible non-random selection is that, within the group of 24 baboons, males and females do not differ in the amount of precipitable cholesterol (consisting of VLDL, IDL, LDL and the faster-floating subspecies of $F^{o}_{1.20}$9-28 lipoproteins), when both groups consume the same diet (Tables 23 and 24). Although males do

possess more IDL and VLDL on the CORN+CS diet and more $F^{o}_{1.20}$9-28 lipoproteins on the COCO+CS diet, these lipoproteins do not provide enough cholesterol mass to balance the higher amount of LDL (S^{o}_{f}5-12 material) the females possess. In any event, a sex difference in LDL may be present among baboons, with females exhibiting more of this lipoprotein class.

Males exhibited a much greater polyunsaturated fat-induced elevation in IDL and VLDL concentrations when switched from the COCO+CS diet to the CORN+CS diet (Table 24, diet-by-sex interaction). That is, while males generally possess lower concentrations than females of these lipoprotein species on the COCO+CS diet, they exhibited higher levels of these lipoproteins while consuming the CORN+CS diet. In fact, female IDL levels were essentially unaffected by the change in dietary fat. Consequently, it appears that IDL and VLDL concentrations of male baboons are sensitive to the type of dietary fat, while the levels of these lipoprotein species among female baboons are generally insensitive to the type of dietary fat.

Experiment I (involving the progeny of baboon sires and dams mated assortatively on the basis of serum cholesterol levels) provided an opportunity to observe the effects of differences in genetic background on lipoprotein levels. As expected, the progeny of hypocholesterolemic sire 959 tended to have lower LDL, IDL and VLDL levels than the progeny of hypercholesterolemic sires. The progeny of

hypocholesterolemic sire 808 did not exhibit mean concentrations of the lipoprotein species significantly lower than those of the progeny of the three hypercholesterolemic sires because, for unknown reasons, one of the five progeny of sire 808 possessed extremely high LDL, IDL and VLDL levels.

LDL particle size distributions, determined by gradient gel electrophoresis (Figures 17, 18 and 19), indicated similarities in the particle sizes of LDL peaks and in the relative heights of major LDL peaks among members of the same family. The relative heights of major LDL peaks, however, varied among families.

3.4. Interrelationships among Concentrations of Lipoprotein Classes

In Experiment I (involving pedigreed progeny of five baboon sires) correlation coefficients among serum concentrations of lipoprotein species were calculated for the entire group of 46 progeny (Tables 7, 10 and 12), the 36 progeny of only the three hypocholesterolemic sires (Tables 15, 16 and 17) and the families of each of the three hypercholesterolemic sires (Tables 15, 16 and 17). The purpose of calculating correlation coefficients among the latter groups was, first of all, to determine whether significant correlations observed among the entire group of 46 progeny merely reflect differences between the progeny of hypocholesterolemic sires and those of hypercholesterolemic sires. As an example, HDL-I and HDL-II concentrations are

positively correlated among the 36 progeny of the three hypercholesterolemic sires. However, when lipoprotein concentrations from the sera of all 46 progeny are used, concentrations of these two lipoprotein species are not correlated. This is because the progeny of hypocholesterolemic sires have lower HDL-I concentrations, but higher HDL-II concentrations. It is of interest to note that in Section 1.4 of the Discussion it was mentioned that among a group of humans consuming an ad lib diet, HDL_{2b} (corresponding to baboon HDL-I) and HDL_{2a} (corresponding to baboon HDL-II) concentrations are positively correlated. The reason for calculating correlation coefficients among the members of each family was to further characterize the differences between each family, providing information for future breeding experiments.

4. Long-term effects of High Levels of Dietary Fat and Cholesterol

The baboons studied in Experiment III (Chapter VII), which were consuming the same diets for six years, provided an opportunity to observe the concentrations of lipoproteins in adult baboons as well as the effects on serum lipoprotein levels of consumption of a high fat and high cholesterol diet for a long period of time.

The baboons in Experiment III exhibited somewhat lower total HDL (and HDL-I) concentrations than the baboons in Experiments I and II, regardless of diet. These lower HDL levels most likely represented an age effect (and not adaptation to the diets consumed), because many of the baboon progeny studied in Experiment I consumed the LARD+CS diet for 3 or more years, but exhibit higher HDL levels than the baboons in Experiment III. Experiment III, however, does not directly demonstrate that HDL levels decrease in the baboon with age because the plasma of each baboon was examined at only one time point.

The baboons in Experiment III also exhibited lower LDL concentrations (than most of the baboons consuming the high cholesterol diets in Experiments I and II), though the mean LDL concentrations were elevated by both saturated fat and by cholesterol. Like the lower HDL concentrations noted above, the generally lower LDL concentrations exhibited by these baboons may indicate an age effect. Another possibility, however, is that the sires used to produce all the

baboons used in Experiment III were hypocholesterolemic. In Experiment I, it was demonstrated that the progeny of hypocholesterolemic sires possess LDL levels of approximately 130 mg/dl (Table 14, progeny of sire 959) and exhibit essentially no $F^{o}_{1.20}9\text{-}28$ lipoproteins. Therefore, it is not possible, with the data available, to determine if LDL (and $F^{o}_{1.20}9\text{-}28$) concentrations decrease with age in the baboon.

5. Applicability of the Baboon Model to Human Lipoprotein Metabolism and Atherosclerosis

The data in this dissertation indicate that baboon lipoproteins have physical-chemical characteristics similar to their human counterparts. In addition to demonstrating comparable flotation rate intervals, chemical and apolipoprotein compositions, the particle size distributions of the HDL and LDL of both species exhibit, under comparable conditions, discrete subpopulations when examined by gradient gel electrophoresis. Among animal species, gradient gel electrophoresis has been applied only to dogs and rats; neither species exhibits lipoprotein subpopulations of the nature observed in baboons and humans.

Baboons exhibit somewhat higher HDL concentrations and lower LDL concentrations than human subjects. The changes in baboon lipoprotein concentrations in response to to dietary manipulation, however, are generally similar in direction to observations made with human subjects. In addition, the statistical correlations among concentrations of lipoprotein classes and subpopulations are remarkably similar between humans and baboons (especially among the progeny of the hypercholesterolemic sires). These data imply that lipoprotein metabolism in baboons and humans may be quite similar. Experiments directly comparing aspects of lipoprotein metabolism in these two species are in progress (125).

Motivation for studying serum lipoproteins and lipoprotein metabolism derives from epidemiological studies wherein LDL has been identified as a positive risk factor (and HDL as a negative risk factor) for coronary heart disease and atherosclerosis in humans (1-7). Similar correlations between serum lipoprotein concentrations and the extent of diet-induced atherosclerosis have also been observed in the baboon (8,22,23,25,26), as well as other animal species (8-14). Therefore, despite unusual sex differences in lipoprotein levels among baboons, the baboon appears to be a good model for human lipoprotein structure and metabolism and the role of lipoproteins in the development of atherosclerosis.

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This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

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