Journal of Clinical Lipidology (2007) 1, 104–141

Journal of Clinical Lipidology

Special Article

The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis and coronary heart disease

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In the past six years the transport of serum lipids via a system of lipoproteins has been described in detail.[1–7] It is our purpose here to describe the physiology of this lipoprotein transport system and the disorders thereof which are found in a variety of metabolic states and in coronary heart disease. The technical methodology for the study of the lipoprotein system is described in detail elsewhere.[3,8–10]

I. Methods and lipoprotein chemistry

The lipoproteins are isolated from serum by the use of the preparative ultracentrifuge. Such isolation is made possible because of two major physico-chemical properties of the lipoproteins, (a) they are substances of high molecular weight (range~60000-10^{11}) and (b) the density of all known lipoproteins is less than that of the serum proteins, such as albumin and the several globulins. Difference in density among the various lipoproteins allows for the segregation of the lipoproteins into three general density ranges. For ultracentrifugal isolation of lipoproteins a set of arbitrary density cutoffs have been employed, at values of 1.063 gms/ml, 1.125 gms/ml, and 1.20 gms/ml. If ultracentrifugation is carried out in a solution of density 1.063 gms/ml (achieved by the addition of sodium chloride to serum), all lipoproteins of densities less than 1.063 gms/ml undergo *flotation* in an intense centrifugal field, whereas the serum proteins and lipoproteins of density greater than 1.063 gms/ml undergo *sedimentation.* In this process all lipoproteins of density 1.04 gms/ml and less (known as the *low-density lipoproteins*) are quantitatively recovered in the top of the ultracentrifuge tube. In the centrifugal run at a solution density of 1.125 gms/ml, two lipoprotein classes, in addition to the above low density group, are quantitatively recovered; those of density 1.05 gms/ml and 1.075 gms/ml. In the centrifugal run at a solution density of 1.20 gms/ml, the remaining class of lipoproteins, of density 1.145 gms/ml, are quantitatively floated to the top of the ultracentrifuge tube.

After isolation, the lipoproteins are quantitatively measured by analytical ultracentrifugation. The flotation of the lipoproteins is visualized and recorded photographically by means of a schlieren optical system. From the photographic record area measurements can be made which are directly proportional to the concentration of lipoproteins being analyzed.[8] (See figure 3).

(a) The ultracentrifugal analysis at a density of 1.063 gms/ml

In this procedure lipoproteins of density 1.04 gms/ml and lower are quantitatively recovered and analyzed. In this low density range of lipoproteins there occurs in the human a series or "spectrum" of lipoproteins of closely spaced flotation rates. Flotation rates for lipoproteins in the analytical ultracentrifuge are measured in terms of a unit known as the Svedberg of flotation, or the S_f unit. One S_f unit represents

This work was supported (in part) by the United States Atomic Energy Commission, The National Heart Institute of the U.S. Public Health Service, and the Life Insurance Medical Research Fund.

1933-2874/$ -see front matter
doi:10.1016/j.jacl.2007.03.001

a flotation rate of 10^{-13} cm/sec/dyne/gram. Since the analytical ultracentrifugation is carried out under specified physical conditions (26°C, solution density of 1.063 gms/ml, 52640 RPM at a radius of 72.5 mm from the center of rotation to the base of the cell), the rate of flotation for a particular lipoprotein species is a physical constant characteristic of that species (*). Thus designation of a lipoprotein by its flotation rate, for example, S_f12, provides positive physico-chemical identification of that lipoprotein. In concentration measurements, the closeness of spacing of lipoprotein flotation rates renders difficult the measurement of the serum level of lipoproteins of each flotation rate. Hence the system has been adopted of measuring the *sum* of concentrations of lipoproteins between arbitrarily chosen flotation rate limits. Thus when one speaks of the concentration of the $S_f0–12$ lipoprotein class, reference is actually being made to the sum of the concentrations of all lipoproteins of flotation rates between the limits S_f0 and S_f12. Similarly one designates the $S_f12–20$, $S_f20–100$, and $S_f100–400$ lipoprotein classes, which with the $S_f0–12$ class make up, in general, the bulk of the low-density lipoprotein "spectrum." There exist low-density lipoproteins of flotation rates between S_f400 and $S_f40{,}000$. However, because of the relatively large variability in concentration of such lipoproteins with respect to food ingestion, systematic measurements have not been made of these lipoproteins. In actual practice flotation rates limits must be measured by taking into account the slowing of lipoprotein flotation as lipoprotein concentration increases. When flotation rate limits have been corrected for such slowing effects, the measured lipoprotein classes are referred to as "Standard" S_f classes to indicate that such corrections have been made. Thus actual reported low density lipoprotein values are in such terms as Standard $S_f0–12$, Standard $S_f12–20$, etc. (Standard S_f is abbreviated $S_f°$ throughout this communication).

b) The ultracentrifugal analysis at a density of 1.125 gms/ml

In this procedure two additional lipoprotein classes are quantitatively measured. One class is of a density of 1.05 gms/ml and is designated as high-density lipoprotein 1, or (HDL-1). The other, of density 1.075 gms/ml, is designated as HDL-2.

c) The ultracentrifugal analysis at a density of 1.20 gms/ml

In this procedure the last additional lipoprotein class is quantitatively measured. This class is of a density of 1.145 gms/ml and is designated as the high-density lipoprotein III class (or HDL−3).

*Except for the effect of lipoprotein concentration itself upon rate (see references 7 and 8.

II. The chemical structure of the serum lipoproteins

In addition to the differences in physical properties among the various lipoproteins, such as density, molecular weight, ultracentrifugal flotation rates, the lipoproteins differ from one another in chemical composition. Such serum lipid constituents as cholesterol, cholesterol esters, phospholipids, fatty acids, and glycerol esters (glycerides) are building blocks of the lipid portion of the various lipoproteins, in the same way that amino acids are the basic structural units of the protein portion of lipoproteins. Studies of the chemical structure of the various lipoprotein classes are reported in extenso by Lindgren, Nichols and Freeman elsewhere.[5,11] The major findings of these workers are presented below.

Ideally it would be desirable to know the complete chemical composition of each lipoprotein class as it exists in the circulating blood stream. This is from the technical point of view not directly achievable.

However, even though some alterations in the lipoproteins may conceivably occur incident to the processes of blood withdrawal, serum preparation, salt solution addition, and prolonged ultracentrifugation, at present, no method superior to the study of in vitro isolated lipoproteins presents itself. By differential preparative ultracentrifugation,[3] serum lipoproteins can be isolated into narrow bands, as ultracentrifugally characterized. However, the narrower the flotation band utilized in isolation procedures, the lower the yield of isolated lipoproteins for chemical analysis. For practical reasons of yield and technique, the present chemical studies have been obtained on relatively broad flotation bands of ultracentrifugally isolated lipoproteins. The following ultracentrifugally isolated lipoprotein bands have been studied:

(1) $S_f°20–400$ —All lipoproteins in this group are of a density lower than 1.007 gms/ml.
(2) $S_f°0–20$ —All lipoproteins of density between 1.007 gms/ml and 1.04 gms/ml are in this group. A part of the HDL−1 lipoprotein (of density 1.05 gms/ml) is present here too. The usual level of HDL−1 lipoproteins is much too low relative to that of the other $S_f°0–20$ lipoproteins to influence appreciably an estimate of $S_f°0–20$ lipoprotein composition.
(3) *The major high density lipoproteins* —The HDL_2 and HDL_3 classes of lipoproteins, of density approximately 1.07 gms/ml and 1.12 gms/ml, respectively, are quantitatively in this fraction. Part of-the HDL-1 lipoprotein, generally present at very low concentration relative to HDL-2 plus HDL_3, is in this group also.
(4) *The total ultracentrifugal lipoprotein "residue"* —This fraction consists of the serum residue that has been freed ultracentrifugally from all the lipoprotein groups listed in (1) to (3) above. Small, and variable, quantities of lipids are present here, especially, and importantly (see below), a high proportion of the unesterified fatty

Table 1 The Lipid Chemical Composition of Isolated Lipoprotein Fractions in Five Humans (*)

Patient	Clinical State	Cholesterol Ester	Unesterified Cholesterol	Glyceride (glycerol esters)	Phospholipid	Unesterified Fatty Acids
The S_f°20–400 class (**)						
1	Apparent clinical health	13.4	9.5	58.4	17.6	1.1
2	Apparent clinical health	12.9	5.4	60.6	20.9	0.3
3	Apparent clinical health	9.3	5.5	64.2	20.4	<0.2
4	Xanthoma tuberosum	32.4	5.4	45.8	16.3	0.2
5	Xanthoma tendinosum	6.9	5.0	53.5	22.7	<0.2
Mean Value . . .		6.2	56.5	19.6		<0.4
The S_f°0–20 class		15.0				
1		51.3	9.7	13.9	24.5	0.6
2		45.0	10.8	16.7	26.5	1.0
3		47.0	11.6	15.9	24.4	1.2
4		45.0	11.8	19.8	22.0	1.2
5		46.1	15.8	10.1	25.9	2.1
Mean Value . . .		46.9	11.9	15.3	24.6	1.2
The Major High Density Lipoproteins						
1		28.6	5.4	14.6	46.5	4.9
2		15.4	6.5	19.8	51.5	6.9
3		19.7	6.3	30.4	38.4	5.2
4		30.3	5.1	20.2	40.2	4.3
5		24.1	8.3	14.1	46.5	6.9
Mean Value . . .		23.6	6.3	19.8	44.6	5.6

(*) Chemical Composition Data are expressed here in percentage of total lipid for each lipid constituent measured in the particular ultracentrifugal fraction. Thus the sum of all percentages for each lipoprotein fraction add up to 100 %.

(**) Analyses reported previously[5] indicate that lipoproteins of S_f°400–40,000 show glycerides to constitute over 75% of the total lipid, the remainder being distributed among cholesterol and phospholipid.

acid of serum, in all probability bound primarily to albumin. Because of the importance of unesterified fatty acids, especially with respect to lipoprotein transformations (see section on Heparinemia), this ultracentrifugal lipoprotein "residue" is considered here along with the various lipoprotein groups. It is expected that possibly additional lipid constituents at very low abundance may be bound to serum proteins in this fraction, but no analyses for such lipids are currently available.

The detailed chemical procedure for extraction and analysis of the lipids of the above-described groups are in reference.[11] Broadly the procedure consists of lipid extraction from the lipoprotein fraction with ethyl ether, followed by lipid separation utilizing silicic acid chromatography[12] patterned after the procedure of Borgstrom.[13] In this modified chromatographic procedure, eluates of 5% chloroform in hexane, 100% chloroform, and 100% methyl alcohol are successively collected, allowing separation of the total lipid sample into the following three categories:

Fraction I — cholesterol esters.
Fraction II — glycerides, unesterified fatty acids, and unesterified cholesterol.
Fraction III — phospholipids.

Quantitative determination of each lipid constituent was made by measurement of infra-red absorption bands characteristic of the particular lipid. In order to determine the lipids present in Fraction II, a method of three component analysis of the absorption bands was employed.[14] The chemical composition data for the lipoprotein fractions in five individuals studied in detail are presented in Table 1. It should be pointed out here that there is no a priori basis for assuming constancy of lipid composition of lipoproteins of a particular ultracentrifugal class from individual to individual in health or in disease. One of the major purposes of this and continuing studies is to determine the extent to which such constancy or lack thereof occurs.

Inspection of the data of Table 1 allows certain generalizations concerning lipoprotein chemical composition to be made:

(1) The phospholipid content is significantly and markedly higher (expressed as percent of total lipid within the lipoprotein) in the major high density lipoprotein group than in either S_f°0–20 or S_f°20–400 lipoprotein classes. Phospholipid is the predominant lipid of the major high density lipoprotein group. The phospholipid content is slightly higher in the S_f°0–20 lipoprotein class than in the S_f°20–400 lipoprotein class.
(2) Cholesterol ester is significantly and markedly higher (expressed as percent of total lipid) in the S_f°0–20 lipoproteins than in either the S_f°20– 400 or the major high density lipoproteins. Cholesterol ester is the predominant lipid constituent of the S_f°0–20 lipropoteins.

(3) Unesterified cholesterol is approximately twice as high (expressed as percentage of total lipid) in the $S_f°0–20$ lipoproteins than in either the $S_f°20–400$ or the major high density lipoprotein group.
(4) The fraction of the cholesterol esterified is less in the $S_f°20–400$ class than in either the $S_f°0–20$ or major high density lipoprotein classes.
(5) The glyceride content is much higher in the $S_f°20–400$ class than in either the $S_f°0–20$ or major high density lipoprotein classes. Within these data it is not possible to ascertain the abundance of mono-, di-, and triglycerides in the total glyceride content of the various lipoprotein classes (*). Glyceride is the predominant lipid constituent of the $S_f°20–400$ lipoproteins.
(6) Unesterified fatty acids: Among the lipoprotein classes, the major high density lipoprotein classes are much higher in percentage content (approximately fivefold) of unesterified fatty acids than are the $S_f°20$ or $S_f°20–400$ lipoprotein classes. Since, in addition, the ultracentrifugal lipoprotein residue contains between 1/3 and 2/3 of the total serum content of unesterified fatty acids (see below) it appears that the two major carriers of unesterified fatty acids are the major high density lipoproteins and the total ultracentrifugal lipoprotein "residue."

The question of extent of variability in lipoprotein composition amongst individuals may be examined in the light of the data of Table 1. The inclusion of xanthoma tuberosum, xanthoma tendinosum, and individuals from the population-at-large in the five subjects analyzed assures that the type of lipoprotein distribution studied includes extremes of difference among such distributions. Thus where constancy of composition is present amongst cases including such extremes, it is reasonable to expect that constancy of composition is a rather general feature. Within the limits of the experimental data of Table 1, it appears that phospholipid shows at the most only a low degree of variability from individual to individual within a single lipoprotein class. This holds for $S_f°0–20$, $S_f°20–400$, or the major high density lipoprotein group.

Cholesterol ester shows very little variation, from individual to individual, for the $S_f°0–20$ lipoprotein class. In the major high density lipoproteins the range of values for the percentage composition of cholesterol ester does suggest that significant variability amongst individuals may exist. In the $S_f°20–400$ lipoprotein class the observed range of percentage composition of cholesterol ester is large, but there are reasons for considering that individual variation is less than the observed values suggest. Case 4 represents a patient with xanthoma tuberosum in whom the distribution of $S_f°20–400$ is much more heavily weighted toward $S_f°20$ than is the case for patients 1, 2, and 3. Since the lipoproteins of flotation rate near $S_f°20$ are higher in cholesterol ester than those of higher flotation rates, the apparently high percentage of cholesterol ester in the lipids of $S_f°20–400$ of case 4 may be largely dependent upon the weighted distribution of lipoproteins in that person. The low cholesterol ester percentage in case 5 may well be related to the unreliability of this particular measurement in case 5, a patient with xanthoma tendinosum, where the yield of $S_f°20–400$ lipoproteins was much lower (because of the nature of the lipoprotein distribution in this disease) than is desirable for satisfactory analysis.

Table 2 Fraction of Unesterified Fatty Acid in the Total Ultracentrifugal Lipoprotein "Residue"

Case	Fraction of Serum Unesterified Fatty Acid in Ultracentrifugal Lipoprotein residue
1	Not analyzed
2	33%
3	39%
4	54%
5	38%

The analytical technique for unesterified cholesterol is such that the apparent variability observed for any of the lipoprotein classes from individual to individual cannot be proved significant within these data.

The glyceride content of $S_f°20–400$ lipoproteins shows little apparent variability. The single value of 45.8%, somewhat below the others in the group is that of case 4, where the same factors mentioned above which might raise the cholesterol ester percentage would operate to reduce the glyceride percentage. The variability of glyceride content of $S_f°0–20$ lipoproteins is considered to be real variability amongst individuals, since none of the errors of the method would account for a range from 10.1 to 19.8% glyceride. It is considered also that the variability in glyceride content in the major high density lipoprotein group reflects significant variation amongst individuals.

The data for unesterified fatty acids are not considered as proving significant individual variability for any particular lipoprotein class from individual to individual.

The total ultracentrifugal lipoprotein "residue" was analyzed in 4 out of the 5 cases described in Table 2. In these four cases, less than 3% of the total lipid content of the serum was found in the ultracentrifugal lipoprotein "residue." The low lipid content of this residue relative to that of the lipoproteins ($S_f°20–400$ and $S_f°0–20$ plus major high density lipoproteins) renders analysis of the lipid distribution of the residue difficult because even low order contamination of the residue with the lipoproteins can provide faulty analysis of such constituents as glyceryl ester, cholesterol ester, phospholipid, and unesterified cholesterol. However, the unesterified fatty acid content of the lipoprotein residue is quite high, such that contamination with the

*The high density lipoproteins show the highest relative abundance of phospholipids and unesterified fatty acids, both being polar lipids. Mono- and di-glyceride would, because of unesterified hydroxyl groups, be somewhat more polar than triglyceride and might hence be relatively more concentrated in the high density lipoproteins than in either $S_f°0–20$ and $S_f°20–400$ lipoproteins. Analyses for mono and di-glycerides are needed to solve this problem.

Table 3a Mean Standard $S_f°0–12$ Lipoprotein Levels

Mean Age	Fasting or non-Fasting	No. of Cases	Mean Standard $S_f°0–12$	Standard Deviation of Distribution	Standard Error of Mean
			(mg/100 ml)	(mg/100 ml)	(mg/100 ml)
MALES					
4.3	Non-fasting	9	312.5	-	-
16.2	Non-fasting	29	263.9	71.0	13.4
25.0	Non-fasting	75	302.0	81.3	9.4
35.1	Non-fasting	358	340.3	87.6	4.6
44.2	Non-fasting	313	363.8	84.4	4.8
44.4	Fasting	412	381.7	91.4	4.5
54.1	Non-fasting	228	367.4	89.4	5.8
54.3	Fasting	431	405.7	92.1	4.5
61.1	Non-fasting	43	360.2	79.1	12.1
63.2	Fasting	187	397.6	89.4	6.5
FEMALES					
5.0	Non-fasting	6	289.9	-	-
16.9	Non-fasting	32	271.0	80.4	15.5
23.5	Non-fasting	86	283.1	71.6	7.6
35.2	Non-fasting	452	304.0	75.7	3.6
44.0	Non-fasting	399	346.1	81.3	4.0
44.0	Fasting	105	351.7	81.1	8.1
53.9	Non-fasting	269	362.9	78.2	4.7
53.8	Fasting	68	388.4	74.8	9.2
61.5	Non-fasting	43	369.2	80.2	12.3
62.8	Fasting	28	426.0	108.0	20.8

Table 3b Mean Standard $S_f12–20$ Lipoprotein Levels

Mean Age	Fasting or Non-Fasting	No. of Cases	Mean Standard $S_f°12–20$	Standard Deviation of Distribution	Standard Error of Mean
			(mg/100 ml)	(mg/100 ml)	(mg/100 ml)
MALES					
4.3	N. F.	9	39.9	9.6	3.4
16.2	N. F.	29	36.5	20.8	3.9
25.0	N. F.	75	48.6	19.0	2.2
35.1	N. F.	358	61.8	28.4	1.5
44.2	N. F.	313	68.3	26.0	1.5
44.4	F.	412	75.7	30.5	1.5
54.1	N. F.	228	69.2	30.5	2.0
54.3	F	431	82.7	31.1	1.5
61.1	N. F.	43	67.2	26.0	4.0
63.2	F	187	78.4	32.5	2.4
FEMALES					
5.0	N. F.	6	37.0	14.8	6.6
16.9	N. F.	32	38.1	18.4	3.3
23.5	N. F.	86	41.9	20.4	2.2
35.2	N. F.	452	53.5	30.9	1.5
44.0	N. F.	399	69.9	33.6	1.7
44.0	F	105	70.1	35.8	3.5
53.9	N. F.	269	89.8	51.5	3.1
53.8	F.	68	92.5	42.3	5.2
61.5	N. F.	43	102.1	53.3	8.2
62.8	F.	28	106.4	48.2	9.2

Table 3c Mean Standard $S_f°20–100$ Lipoprotein Levels

Mean Age	Fasting or Non-Fasting	No. of Cases	Mean Standard $S_f°20–100$	Standard Deviation of Distribution	Standard Error of Mean
			(mg/100 ml)	(mg/100 ml)	(mg/100 ml)
MALES					
4.3	N.F.	9	68.1	19.5	6.9
16.2	N.F.	29	66.5	28.7	5.4
25.0	N.F.	75	86.5	41.2	4.8
35.1	N.F.	358	101.0	48.8	2.6
44.2	N.F.	313	108.9	47.9	2.7
44.4	F.	412	105.5	56.2	2.8
54.1	N.F.	228	111.8	56.4	3.7
54.3	F.	431	106.2	57.1	2.8
61.1	N.F.	43	104.6	45.0	6.9
63.2	F.	187	100.1	53.8	3.9
FEMALES					
5.0	N.F.	6	64.7	75.5	33.8
16.9	N.F.	32	52.4	24.2	4.4
23.5	N.F.	86	49.1	26.2	2.8
35.2	N.F.	452	63.6	34.3	1.6
44.0	N.F.	399	78.4	39.9	2.0
44.0	F.	105	64.1	37.6	3.7
53.9	N.F.	269	98.6	45.7	2.8
53.8	F.	68	82.9	44.1	5.4
61.5	N.F.	43	131.5	69.4	10.7
62.8	F.	28	105.3	65.9	12.7

Table 3d Mean Standard $S_f°100–400$ Lipoprotein Levels

Mean Age	Fasting or Non-Fasting	No. of Cases	Mean Standard $S_f°100–400$	Standard Deviation of Distribution	Standard Error of Mean
			(mg/100 ml)	(mg/100 ml)	(mg/100 ml)
MALES					
4.3	N.F.	9	24.2	18.1	6.4
16.2	N.F.	29	28.2	23.1	4.4
25.0	N.F.	75	43.0	30.9	3.6
35.1	N.F.	358	73.5	79.5	4.2
44.2	N.F.	313	82.9	77.1	4.4
44.4	F.	412	49.5	58.2	2.9
54.1	N.F.	228	75.9	72.6	4.8
54.3	F.	431	44.4	48.8	2.4
61.1	N.F.	43	67.4	54.0	8.3
63.2	F.	187	42.1	46.8	3.4
FEMALES					
5.0	N.F.	6	27.3	19.9	8.9
16.9	N.F.	32	14.1	11.2	2.0
23.5	N.F.	86	15.9	13.7	1.5
35.2	N.F.	452	25.8	27.1	1.3
44.0	N.F.	399	39.0	41.0	2.1
44.0	F.	105	23.1	19.9	2.0
53.9	N.F.	269	51.5	44.1	2.7
53.8	F.	68	29.8	19.9	2.4
61.5	N.F.	43	67.2	52.2	8.1
62.8	F.	28	42.8	34.0	6.5

Table 4 Mean Standard $S_f°$ 12–400 Lipoprotein Levels

Mean Age	Fasting or Non-Fasting	No. of Cases	Mean Standard $S_f°$ 12–400	Standard Deviation of Distribution	Standard Error of Mean
			(mg/100 ml)	(mg/100 ml)	(mg/100 ml)
MALES					
4.3	N.F.	9	132.2	—	—
16.2	N.F.	29	131.3	63.6	12.4
25.0	N.F.	75	178.1	83.8	9.7
35.1	N.F.	358	236.1	135.0	7.2
44.2	N.F.	313	259.8	128.1	7.2
44.4	F.	412	230.7	117.3	5.8
54.1	N.F.	228	256.9	135.5	9.0
54.3	F.	431	233.0	112.9	5.5
61.1	N.F.	43	239.2	100.1	15.5
63.2	F.	187	220.6	109.1	8.1
FEMALES					
5.0	N.F.	6	129.0	—	—
16.9	N.F.	32	104.8	43.5	9.6
23.5	N.F.	86	107.1	50.2	5.4
35.2	N.F.	452	142.9	77.7	3.6
44.0	N.F.	399	187.3	96.5	4.9
44.0	F.	105	157.2	80.2	7.8
53.9	N.F.	269	239.7	116.5	7.2
53.8	F.	68	205.2	88.9	10.8
65.1	N.F.	43	300.8	146.9	22.6
62.8	F.	28	254.2	125.7	24.2

lipoproteins (the maximal extent of which can be estimated) cannot possibly have appreciably influenced the result for unesterified fatty acid. The findings for unesterified fatty acid in the lipoprotein "residue" indicate that a high proportion of the total serum unesterified fatty acid is present there. For the four cases analyzed the actual fraction of serum unesterified fatty acid found in the lipoprotein residue are given in Table 2.

Considerations, including the chemical composition data, indicate that neither the *total* lipoprotein concentration nor the distribution of lipoproteins amongst the various classes can be inferred from a chemical determination such as that of the serum cholesterol. From our best estimates of lipid-protein ratios within the lipoproteins, it appears that lipids represent 85–90% of the lipoprotein for the $S_f°20–$

Table 5 The HDL_1 Concentration in males and females as a function of chronological age (**)

Age Group (years)	No. of Subjects	Mean HDL_1 (mg/100 ml)	Standard Deviation of Distribution (mg/100 ml)	Standard Error of Mean (mg/100 ml)
MALES				
18–19	15	21.8	3.6	1.0
20–29	28	21.6	7.7	1.5
30–39	85	17.5	7.9	0.9
40–49	71	18.2	9.8	1.2
50–59	55	19.0	8.5	1.2
60–69	16	19.6	8.5	2.2
FEMALES				
18–19	22	17.6	6.2	1.4
20–29	28	15.8	5.8	1.1
30–39	157	14.2	6.5	0.5
40–49	156	15.6	6.0	0.5
50–59	80	17.4	5.5	0.6
60–69	17	17.5	12.2	3.1

(*) Population sample in the United States.

Table 6 The HDL_2 Concentration in males and females as a function of chronological age

Age Group (years)	No. of Subjects	Mean HDL_2 (mg/100 ml)	Standard Deviation of Distribution (mg/100 ml)	Standard Error of Mean (mg/100 ml)
MALES				
18–19	15	82.5	18.7	5.0
20–29	28	59.6	26.0	5.0
30–39	85	58.8	31.4	3.4
40–49	71	61.3	31.5	3.8
50–59	55	63.3	39.6	5.4
60–69	16	74.3	36.9	9.5
FEMALES				
18–19	22	109.8	45.2	9.9
20–29	28	141.3	64.0	12.3
30–39	157	102.4	45.9	3.7
40–49	156	103.1	43.5	3.5
50–59	80	99.9	48.9	5.5
60–69	17	88.0	37.8	9.5

Table 7 The HDL_3 concentration in males and females as a function of chronological age

Age Group (years)	No. of Subjects	Mean HDL_3 (mg/100 ml)	Standard Deviation of Distribution (mg/100 ml)	Standard Error of Mean (mg/100 ml)
MALES				
18–19	15	203.0	32.5	8.7
20–29	27	195.5	36.7	7.2
30–39	85	169.3	47.9	5.2
40–49	71	178.1	42.0	5.0
50–59	55	181.9	51.7	7.0
60–69	16	182.3	42.1	10.9
FEMALES				
18–19	22	204.5	30.7	6.7
20–29	28	222.6	37.1	7.1
30–39	157	192.4	44.0	3.5
40–49	156	203.2	38.8	3.1
50–59	80	204.3	36.6	4.1
60–69	17	180.6	38.1	9.5

Table 8 Low-Density Lipoprotein Interclass Relationships

Lipoprotein classes	Pearson r	Significance Test
40–49 *year old Males* (*)	(*Number of subjects* = 309)	
$S_f°0–12$ vs. $S_f°12–20$	+0.54	$P < 0.01$
$S_f°0–12$ vs. $S_f°20–100$	+0.08	N.S.
$S_f°0–12$ vs. $S_f°100–400$	+0.05	N.S.
$S_f°12–20$ vs. $S_f°20–100$	+0.44	$P < 0.01$
$S_f°12–20$ vs. $S_f°100–400$	+0.22	$P < 0.01$
$S_f°20–100$ vs. $S_f°100–400$	+0.74	$P < 0.01$
30–39 *year old Females* (*)	(*Number of subjects* = 446)	
$S_f°0–12$ vs. $S_f°12–20$	+0.58	$P < 0.01$
$S_f°0–12$ vs. $S_f°20–100$	+0.35	$P < 0.01$
$S_f°0–12$ vs. $S_f°100–400$	+0.30	$P < 0.01$
$S_f°12–20$ vs. $S_f°20–100$	+0.59	$P < 0.01$
$S_f°12–20$ vs. $S_f°100–400$	+0.38	$P < 0.01$
$S_f°20–100$ vs. $S_f°100–400$	+0.74	$P < 0.01$

(*) All correlations recorded here are for non-fasting samples.

400 class, 75–80% of the lipoprotein for the $S_f°0–20$ class, and 40–50% of the major high density lipoproteins. Combining these data with the fraction of the lipid represented by cholesterol (esterified plus unesterified) obtained from the mean values of Table 1, it is arrived at that cholesterol represents 19% of $S_f°20–400$ lipoproteins, 45% of $S_f°0–20$ lipoproteins and 13% of the major high density lipoproteins. Thus unless one knows the lipoprotein distribution, the total lipoprotein concentration cannot be estimated from a cholesterol measurement. Further extensive studies have revealed that at identical serum cholesterol levels, lipoprotein distributions may be grossly different, one individual showing a great preponderance of $S_f°20–400$ lipoproteins, with another showing a great preponderance of $S_f°0–20$ lipoproteins. This latter fact, crucial in several metabolic studies, in atherosclerosis, and coronary heart disease (see below), cannot be discovered from a chemical determination such as a serum cholesterol measurement. Similar consideration would apply for chemical determinations of any other single lipid constituent.

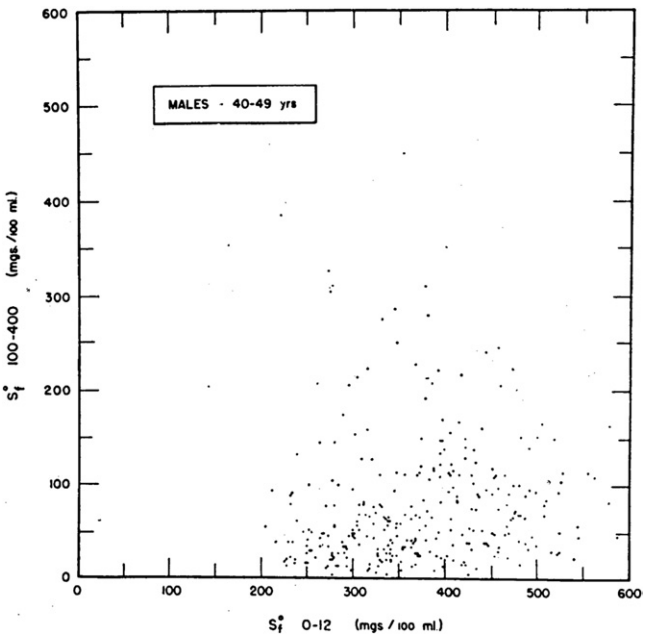

Figure 1 Scattergram showing the Relationship of $S_f°0–12$ Lipoprotein Levels to $S_f°$ 100–400 Lipoprotein Levels in a representative sample of the United States population of 40–49 year old males (309 cases).

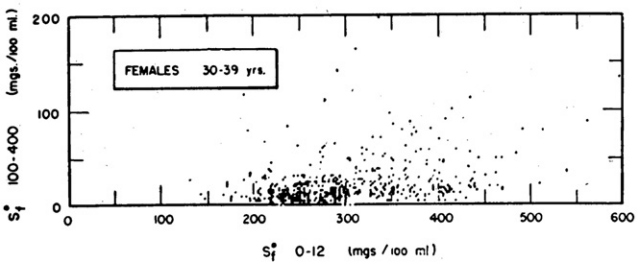

Figure 2 Scattergram showing the Relationship of $S_f°0–12$ Lipoprotein Levels to $S_f°100–400$ Lipoprotein Levels in a representative sample of the United States Population of 30–39 year old females (446 cases).

Table 9 High-Density Lipoprotein-Interclass-Relationships

Age Group	No. of Subjects	Pearson r	Significance Test
HDL_1 VERSUS HDL_2			
Males			
30–39 yrs.	83	+0.03	N.S.
40–49 yrs.	69	−0.20	N.S.
50–59 yrs.	53	−0.14	N.S.
Females			
30–39 yrs.	155	−0.11	N.S.
40–49 yrs.	154	−0.28	p = 0.01
50–59 yrs.	78	−0.27	N.S.
HDL_1 VERSUS HDL_3			
Males			
30–39 yrs.	83	+0.29	p = 0.01
40–49 yrs.	69	−0.07	N.S.
50–59 yrs.	53	+0.34	p = 0.05
Females			
30–39 yrs.	155	−0.04	N.S.
40–49 yrs.	154	+0.04	N.S.
50–59 yrs.	78	0.00	N.S.
HDL_2 VERSUS HDL_3			
Males			
30–39 yrs.	83	+0.38	p = 0.01
40–49 yrs.	69	+0.17	N.S.
50–59 yrs.	53	+0.22	N.S.
Females			
30–39 yrs.	155	+0.17	p = 0.05
40–49 yrs.	154	−0.09	N.S.
50–59 yrs.	78	+0.34	p = 0.05

Table 10 High Density Lipoprotein - Low Density Lipoprotein Correlations

Age Group	No. of Subjects	Versus S_f°0–12	Significance Test	Versus S_f°12–20	Significance Test	Versus S_f°20–100	Significance Test	Versus S_f°100–400	Significance Test
FOR HDL_1 VERSUS THE FOUR LOW DENSITY LIPOPROTEIN CLASSES									
Males									
30–39 yrs.	83	+0.18	N.S.	+0.15	N.S.	+0.21	p = 0.05	+0.24	0.05 > P > 0.01
40–49 yrs.	69	−0.08	N.S.	−0.14	N.S.	+0.42	p = 0.01	+0.56	p = 0.01
50–59 yrs.	53	+0.07	N.S.	+0.03	N.S.	+0.33	0.05 > P > 0.01	+0.27	p = 0.05
Females									
30–39 yrs.	155	+0.19	0.05 > P > 0.01	+0.03	N.S.	+0.20	p = 0.01	+0.20	p = 0.01
40–49 yrs.	154	+0.16	p = 0.05	−0.02	N.S.	+0.05	N.S.	+0.12	N.S.
50–59 yrs.	78	+0.15	N.S.	+0.10	N.S.	+0.10	N.S.	+0.17	N.S.
FOR HDL_3 VERSUS THE FOUR LOW DENSITY LIPOPROTEIN CLASSES									
Males									
30–39 yrs.	83	−0.36	p = 0.01	−0.24	0.05 > P > 0.01	−0.24	0.05 > P > 0.01	−0.17	N.S.
40–49 yrs.	69	−0.28	0.05 > P > 0.01	+0.07	N.S.	−0.22	N.S.	−0.25	0.05 > P > 0.01
50–59 yrs.	53	−0.25	p = 0.05	−0.19	N.S.	−0.12	N.S.	+0.04	N.S.
Females									
30–39 yrs.	155	−0.19	0.05 > P > 0.01	−0.14	N.S.	−0.26	p = 0.01	−0.21	p = 0.01
40–49 yrs.	154	−0.11	N.S.	−0.01	N.S.	−0.09	N.S.	−0.17	0.05 > P > 0.01
50–59 yrs.	78	−0.31	p = 0.01	−0.17	N.S.	−0.30	p = 0.01	−0.37	p = 0.01
FOR HDL_3 VERSUS THE FOUR LOW DENSITY LIPOPROTEIN CLASSES									
Males									
30–39 yrs.	83	−0.08	N.S.	−0.01	N.S.	−0.11	N.S.	−0.09	N.S.
40–49 yrs.	69	−0.02	N.S.	−0.12	N.S.	−0.11	N.S.	−0.16	N.S.
50–59 yrs.	53	−0.07	N.S.	−0.10	N.S.	−0.09	N.S.	−0.04	N.S.
Females									
30–39 yrs.	155	0.00	N.S.	−0.01	N.S.	−0.11	N.S.	−0.04	N.S.
40–49 yrs.	154	+0.01	N.S.	0.00	N.S.	−0.06	N.S.	+0.05	N.S.
50–59 yrs.	78	+0.05	N.S.	−0.20	N.S.	−0.07	N.S.	−0.08	N.S.

III. The biology of the distribution of the various lipoproteins in populations

It is of great interest that the human population (at least in the USA) shows considerable heterogeniety with respect to the distribution of concentrations of the various lipoprotein classes habitually present in the serum. Short term variability of the distribution of concentrations of the various lipoproteins in a single individual is of a considerably smaller magnitude. The prominent role of age and sex in

Table 11 Low Density Lipoprotein Levels in Xanthoma Tendinosum

	S_f°0–12 mg/100 ml	S_f°12–20 mg/100 ml	S_f°20–100 mg/100 ml	S_f°100–400 mg/100 ml
Mean Lipoprotein Levels in Patients with Xanthoma Tendinosum (18 subjects)	793	150	128	36
Range of levels in Xanthoma Tendinosum	452–1196	49–372	36–396	0–85
Mean Lipoprotein levels in matched controls	336	65	92	56
Difference (*) in mean levels in standard score units	+5.3	+3.1	+0.8	−0.4
Significance Test on Difference of Means	$P \ll 0.01$	$P \ll 0.01$	P = 0.01	P = 0.01

(*) Differences = Mean for Study Group − Mean for Matched Controls.

Table 12 High Density Lipoprotein Levels in Xanthoma Tendinosum

	HDL_1 mg/100ml	S. D.*	HDL_2 mg/100ml	S. D.	HDL_3 mg/100ml	S. D.
Mean Levels in Xanthoma Tendinosum (9 cases)	25.8	6.3	14.9	14.4	149.8	28.3
Range of levels in xanthoma tendinosum	18–36		3–51		94–179*	
Mean levels in matched controls	17.1		88.3		193.2	
Difference in Means in mg/100ml (xanthoma tendinosum-matched controls)	+8.7		−73.4		−43.4	
Significance Test on Difference Between Means	$p = 0.01$		$p < 0.01$		$p < 0.01$	

*S. D. refer to Standard Deviation of the distribution throughout all tables in this communication.

determination of serum lipoprotein distributions becomes apparent when studies are made of groups of individuals of both sexes over a large range of ages. Lipoprotein measurements for population samples (USA) are available from the range from early childhood up through the seventh decade of life.[9] The mean low density lipoprotein values and the standard deviation of the distributions for both sexes as a function of age are given in Table 3 (Table 3a 3b 3c 3d) and Table 4.

Of note is the very small difference, if any significant difference exists, observed between males and females up to the age of 15 years for all four low density lipoprotein classes. Thereafter males and females diverge sharply in terms of the further history of the mean levels of all four low-density lipoprotein classes with increasing age, the males remaining consistently higher than the females until the age range between 45 and 65 years, during which time mean levels for all four lipoprotein classes in the females cross those of the males and then become higher. Much has been commented upon in the literature concerning hormonal changes at "the climateric" accounting for serum lipid alterations. It should be pointed out that the serum lipoprotein data do not support a concept of any short term period of the order of 2–5 years as a "climateric" period in this regard, since the lipoprotein means indicate a continuous type of alteration in the 40 year interval following the immediate postpuberal period.

Similar measurements are available for the three classes of high-density lipoproteins, HDL_1, HDL_2, and HDL_3.[10] The means and the standard deviations of the distributions are presented in Tables 5, 6, and 7. It is worthy of note that, while sex and ageing differences are evident for these lipoprotein classes as well as for the above-described low-density lipoproteins, the age and sex effects do not parallel the trends seen for the low-density lipoproteins, suggesting that the same metabolic basis does not, in all probability, underlie the age changes in these two major groups of lipoproteins.

IV. Lipoproteins' interclass relationships

It is self-evident that a major objective in the study of the population trends in lipoprotein concentrations with age and sex is an understanding of the factors, metabolic or otherwise, which underlie such trends. One step in this direction is a determination of the extent to which the levels of the various lipoprotein classes are interrelated. Thus for any two classes of lipoproteins the levels of which are either strongly positively or strongly negatively interrelated quantitatively, it is reasonable to infer the existence of certain common factors in the control of the serum levels. Two representative groups have been chosen for correlation studies of the

Table 13 Low Density Lipoprotein Levels in Xanthoma Tuberosum

	$S_f°0–12$ mg/100 ml	$S_f°12–20$ mg/100 ml	$S_f°20–100$ mg/100 ml	$S_f°100–400$ mg/100 ml
Mean Lipoprotein Levels in Patients with Xanthoma Tuberosum (23 patients)	206	128	616	650
Range of levels in Xanthoma Tuberosum	105–403	74–242	314–1832	74–2804
Mean Lipoprotein Levels in Matched Controls	358	74	105	72
Difference in Mean Levels in Standard Score Units	−1.9	+1.8	+9.1	+7.9
Significance Test on Difference Between Means	$p < 0.01$	$p < 0.01$	$p \ll 0.01$	$p \ll 0.01$

Table 14 High Density Lipoproteins in Xanthoma Tuberosum

	HDL_1 mg/100 ml	S. D.	HDL_2 mg/100 ml	S. D.	HDL_3 mg/100 ml	S. D.
Mean high density Lipoprotein Levels in xanthoma tuberosum (16 cases)	25.6	20.4	38.5	31.8	165.9	41.0
Range of levels in xanthoma tuberosum	3–88		0–124		85–249	
Mean levels in matched controls	17.4		75.6		185.2	
Difference in means in mg/100 ml (xanthoma tuberosum-matched controls)	+8.2		−37.1		− 19.3	
Significance test on the difference between means	N.S (*)		$P < 0.01$		N.S.	

(*) N.S. means not significant at the 5% level or below. This notation is used throughout.

low density lipoproteins, 40–49 year old males and 30–39 year old females. The Pearson product-moment correlations measured between all possible pairs of low density lipoprotein classes are presented in Table 8 for 40–49 year old males and for 30–39 year old females.

Inspection of the correlation coefficients in Table 8 for both sexes shows the highest interclass relations to exist for lipoprotein classes most closely related in flotation rates, that is, for $S_f°0–12$ vs $S_f12–20$, $S_f°12–20$ vs $S_f°20–100$, and $S_f°20–100$ vs $S_f°100–400$. The magnitude of these inter class correlations is not significantly different between the two sexes. All other interclass relationships are of a lower order. For $S_f°0–12$ vs $S_f°20–100$, $S_f°0–12$ vs $S_f°100–400$, and $S_f°12–20$ vs $S_f°100–400$ there appears to be a higher interrelationship in females than in males. It is evident from the magnitudes of the various correlation coefficients that a complete description of the low-density lipoprotein levels in an individual requires a measurement of all four low-density lipoprotein classes.

For both males and females the correlation between $S_f°0–12$ and $S_f°100–400$ lipoprotein levels is the lowest, indicating a high degree of independence of the metabolic factors involved in the control of the serum levels of these two classes. This independence is well demonstrated in the scattergrams of Figures 1 and 2 for the $S_f°0–12$ and $S_f°100–400$ lipoproteins in 40–49 year old males and 30–39 year old females, respectively.

(b) High density lipoproteins

The Pearson product-moment correlations measured between all possible pairs of high-density lipoprotein classes are presented in Table 9 for several age categories in both sexes.

It is evident, from evaluation of the very low correlation coefficients for the interrelationships of HDL_1, HDL_2, and HDL_3 that the factors involved in the control of the serum levels of these three lipoprotein classes in populations are largely independent of each other. This does not preclude the existence of subsegments of the overall population in which high interclass relationships may exist, either of positive or negative value. However, the net effect of any such, as yet unidentifiable subsegments, is to produce the low correlations observed unidentifiable.

(c) High density lipoproteins versus low density lipoproteins

The Pearson product-moment correlations measured between all possible pairs of high density lipoproteins and low

Table 15 Low Density Lipoprotein Levels in Xanthelasma

	$S_f°0–12$ mg/100 ml	$S_f°12–20$ mg/100 ml	$S_f°20–100$ mg/100 ml	$S_f°100–400$ mg/100 ml
Mean lipoprotein levels in patients with xanthelasma (43 cases)	444	112	105	54
Range of levels in xanthelasma	258–979*	36–405	36–267	7–179
Mean lipoprotein levels in matched controls	352	76	96	54
Difference in mean levels in Standard score units	+1.1	+1.2	+0.2	0.0
Significance test on the difference between means	$P < 0.01$	$P < 0.01$	N.S.	N.S.

(*) It is of interest to note that the two cases having $S_f°0–12$ levels below 300 mg/100 ml showed the two highest $S_f°12–20$ levels. (Case 1, $S_f°0–12 = 258$; $S_f°12–20 = 405$; Case 2, $S_f°0–12 = 284$; $S_f°12–20 = 273$).

Table 16 High Density Lipoproteins in Xanthelasma

	HDL_1 mg/100 ml	S. D.	HDL_2 mg/100 ml	S. D.	HDL_3 mg/100 ml	S. D.
Mean high density lipoprotein level in xanthelasma (5 cases)	19.4	4	64.0	59	204	45
Range of levels in xanthelasma	12–23		24–169		132–258	
Mean levels in matched controls	15.8		91.0		191.0	
Difference in means in mg/100 ml (xanthelasma-matched controls)	+2.7		−27.0		+13.0	
Significance test on the difference between means	N.S.		N.S.		N.S.	

density lipoproteins are presented in Table 10 for several age categories in both sexes.

The HDL_1 class shows moderate positive correlation with both the $S_f°20–100$ and $S_f°100–400$ density lipoproteins in the male sex. These relationships, although in the same direction in the female are very low and only of borderline significance from the available data. The relationship between HDL_1 and $S_f°0–12$ and $S_f°12–20$ in the male can in no case be proven significant, although a very weak positive correlation between HDL_1 and $S_f°0–12$ appears to exist for the female sex. In any event, all the measured correlations between HDL_1 and the four low density lipoproteins are of such low order as to indicate, for the population as a whole, the factors controlling HDL_1 lipoprotein levels are largely independent of those controlling the low density lipoprotein levels.

The HDL_2 serum lipoprotein levels show significant, but low-order *negative* correlations with $S_f°0–12$, $S_f°100$, and $S_f°100–400$ lipoprotein levels, respectively, in both sexes. Probably a low negative correlation exists between HDL_2 lipoproteins and $S_f°12–20$ as well, although significance could not be proved in most individual age and sex categories. The HDL_2 relationships with the various low density lipoproteins represent the only appreciable *negative* correlations discovered for the entire lipoprotein spectrum. Even this negative correlation is of very low order, such that for the population at large it can be stated that the factors controlling serum HDL_2 lipoprotein levels are largely independent of those controlling the levels of the four low density lipoproteins.

The HDL_3 serum lipoprotein levels cannot, within these data, be demonstrated to show any significant correlations with any of the four low density lipoprotein classes. It appears, therefore that the factors controlling HDL_3 lipoprotein levels in the population at large are essentially completely independent of those controlling the levels of the low-density lipoprotein classes.

V. The serum lipoproteins in various metabolic states

A variety of metabolic states in which lipid metabolic alterations were known to exist or suspected to exist have been investigated with respect to the serum transport of lipoproteins.[15–21] Such studies have revealed that there is a considerable diversity in the types of lipoprotein transport derangement present in certain metabolic disturbances, whereas for a particular metabolic disorder the lipoprotein transport pattern is reasonably specific. One important corollary finding of such studies is that a classification of metabolic states by the older chemical determination of serum cholesterol is wholly inadequate for satisfactory description of the lipid metabolic disturbance present. Thus hypercholesteremia must be regarded as simply a conglomeration of several disorders, unrelated not only because of etiologic basis, but also unrelated in terms of the actual lipoprotein transport aberration present. This will become apparent through the consideration of the low and high

Table 17 Low Density Lipoprotein Levels in the Nephrotic State

	$S_f°0–12$ mg/100 ml	$S_f°12–20$ mg/100 ml	$S_f°20–100$ mg/100 ml	$S_f°100–400$ mg/100 ml
Mean lipoprotein levels in patients in nephrotic state (13 cases)	787	236	583	355
Range of levels in nephrotic state	455–1290	85–401	213–1219	37–748
Mean lipoprotein levels in matched controls	276	41	69	37
Difference in mean levels in standard score units	+6.3	+9.8	+14.7	+11.0
Significance test on the difference between means	$p \ll 0.01$	$p \ll 0.01$	$p \ll 0.01$	$p \ll 0.01$

Table 18 High Density Lipoproteins in the Nephrotic State

	HDL_1 mg/100 ml	S. D.	HDL_2 mg/100 ml	S. D.	HDL_3 mg/100 ml	S. D.
Mean high density lipoprotein level in the nephrotic state (5 cases)	33.2	28.3	16.0	26.0	157.0	126.2
Range of levels in the nephrotic state	5–82		0–67		21–396	
Mean levels in matched controls	20.6		71.2		186.5	
Difference in means in mg/100 ml (nephrotic state-matched controls)	+12.6		−55.2		−29.5	
Significance test on the difference between means	N.S.		$p < 0.01$		N.S.	

density lipoprotein findings in the various metabolic states described below.

1. Xanthoma tendinosum

(a) Low density lipoproteins

A group of 18 patients with xanthomatous lesions involving the tendons was studied. Some of the patients reported their lesions had been present since childhood. For purposes of comparison of the levels in xanthoma tendinosum with clinically healthy individuals a control series matched (*) by age and sex was prepared from a large series of random clinically healthy individuals. A useful approach to consideration of the extent of difference of lipoprotein levels between patients with xanthoma tendinosum and the control group is that utilizing standard scores. The standard scores of difference in means between the disease and matched control populations is equal to the difference in means divided by the standard deviation of the measurement. The series of xanthoma tendinosum constituted 11 males ranging in age from 27–50 years of age and 7 females ranging in age from 14–52 years of age. The values for the four standard low density lipoprotein classes are given in Table 11.

In xanthoma tendinosum the increase in low density lipoprotein levels is greatest in the $S_f°0–12$ class, next in the $S_f°12–20$ class, and least in the $S_f°20–100$ class. The $S_f°100–400$ lipoprotein level is slightly, but significantly, lower in xanthoma tendinosum than in matched controls.

(b) High Density Lipoproteins

High density lipoprotein levels are available for 9 patients with xanthoma tendinosum, including 4 males and 5 females. The mean levels for the three high-density lipoprotein classes are presented in Table 12, together with a significance test for the difference between the means in xanthoma tendinosum and in matched controls.

The HDL_1 level in xanthoma tendinosum shows a small, but statistically significant, elevation over that in matched controls. The measurement problem for HDL_1 in the presence of exceedingly high $S_f°0–12$ levels is greater than elsewhere, and hence in this special case, the elevation may be subject to error of a systematic character.

The HDL_2 lipoproteins are markedly, and highly significantly, reduced in xanthoma tendinosum. The extent of reduction is secondary only to that observed in chronic biliary obstruction (see below). It is of interest that in spite of the very low interrelationship of the $S_f°0–12$ versus HDL_2 (see Table 10) in the population at large, this special group, xanthoma tendinosum, shows a marked reduction in HDL_2 accompanying the marked elevation in $S_f°0–12$. It is entirely possible that individuals with the same type of metabolic disorder, but less marked, are admixed into the population at large and may help account for what low order inverse correlation does exist between HDL_2 and $S_f°0–12$ lipoproteins.

The HDL_3 lipoproteins are also significantly reduced in xanthoma tendinosum. The extent of reduction is not however as marked as is the case for the HDL_2 lipoproteins. Again this is of interest in view of the extremely low order of a possible inverse correlation (see Table 10) between HDL_3 and $S_f°0–12$ lipoproteins in the population at large.

2. Xanthoma tuberosum

(a) Low density lipoproteins

A group of 23 patients with xanthomatous lesions of the skin was studied. Characteristic sites of the lesions in this group were (1) extensor surfaces of the elbows, (2) buttocks, (3) extensor aspect of the knees, (4) the hands, especially the volar surfaces, (5) over the ankle malleoli, especially laterally. In contrast to the patients with xan-

*Throughout this comunication, matched control values for each study case represent the mean for clinically healthy individuals of the same age and sex as the study case.

Table 19 Low Density Lipoproteins in Chronic Biliary Obstruction

	S_f°0–12 mg/100 ml	S_f°12–20 mg/100 ml	S_f°20–100 mg/100 ml	S_f°100–400 mg/100 ml
Mean lipoprotein levels in patients with biliary obstruction (6 cases)	910	1053	1265	49
Range of levels in biliary obstruction	579–1550	575–2119	150–3472	0–114
Mean lipoprotein levels in matched controls	346	70	78	39
Difference in mean levels in standard score units	+7.0	+29.2	+29.7	+0.3
Significance test on the difference between means	$p \ll 0.01$	$p \ll 0.01$	$p \ll 0.01$	N.S.

thoma tendinosum, none of the patients with xanthoma tuberosum reported the presence of lesions in childhood. The series of xanthoma tuberosum for which low density lipoprotein levels are presented constituted 17 males ranging in age from 27 to 59 years and 6 females ranging in age from 44 to 60 years. The low density lipoprotein levels for xanthoma tuberosum and for matched controls are given in Table 13.

In xanthoma tuberosum the increase in low-density lipoprotein levels is greatest in the S_f°20–100 class and in the S_f°100–400 class. The S_f°12–20 lipoprotein level is significantly increased, but to a much lesser extent than the S_f°20–100 or S_f°100–400 classes. However, the S_f°0–12 lipoprotein level is significantly *lower* than in the matched controls.

The age-sex distribution of cases of xanthoma tuberosum shows that in this series 14 of the 17 males were under the age of 50 years, while 5 of the 6 females were over the age of 50 years. A chi-square test indicates that this distribution could occur by chance alone in less than one in one hundred times. It is significant therefore that in males xanthoma tuberosum develops at a much younger age than in females. An estimate based upon the patient's statement of age of onset of visible lesions leads to the same conclusion.

(b) High density lipoproteins

High density lipoprotein levels are available for 16 patients with xanthoma tuberosum, including 11 males and 5 females. The mean levels for the three high density lipoprotein classes are presented in Table 14.

The HDL_1 levels cannot be proven to be significantly altered in xanthoma tuberosum within the framework of the available data. The HDL_2 levels are significantly lower than for matched controls, but the extent of lowering is less than that observed in the metabolic disturbance which characterizes xanthoma tendinosum. The HDL_3 level cannot be proven significantly lower than that for matched controls, within the available data.

3. Xanthelasma

(a) Low density lipoproteins

A group of 43 patients with xanthelasma (xanthoma palpebrarum) was studied. These patients represent a group referred for study because of xanthelasma rather than for such diseases as coronary heart disease which did co-exist in some cases. None of these patients showed frank xanthomatous lesions in sites other than the eyelids. The series of xanthelasma patients constituted 11 males, ranging in age from 37 years to 68 years and 32 females ranging in age from 33 years to 70 years. The values for the four low density lipoprotein classes are given in Table 15.

In xanthelasma an increase in low-density lipoprotein levels is noted in both the S_f°0–12 and S_f°12–20 classes. The extent of lipoprotein level elevation above the matched control series is essentially the same (on the standard score basis) for the S_f°0–12 and S_f°12–20 classes. Xanthelasma is characterized by the absence of a significant deviation in lipoprotein levels from those of the matched controls either in the S_f°20–100 or S_f°100–400 classes.

(b) High density lipoproteins

High density lipoprotein levels are available only for 5 patients with xanthelasma (2 males, 3 females). The mean levels for the high-density lipoprotein classes are presented in Table 16.

No significant deviation in mean levels for HDL_1, HDL_2, or HDL_3 from those in matched controls can be demonstrated for xanthelasma. However, the lack of demonstrable significant difference here may well be the result of the paucity of cases studied. Certainly this group requires further evaluation of high density lipoprotein levels.

Table 20 High Density Lipoproteins in Chronic Biliary Obstruction

	HDL_1 mg/100 ml	S. D.	HDL_2 mg/100 ml	S. D.	HDL_3 mg/100 ml	S. D.
Mean high density lipoprotein levels in chronic biliary obstruction (5 cases)	8.4	11.0	0.6	—	16.6	25.8
Range of levels in chronic biliary obstruction	9–27		0–3		0–62	
Means in matched controls	15.4		101		195.8	
Difference in means in mg/100 ml (chronic biliary obstruction-matched controls)	−7.0		−100.4		−179.2	
Significance test on the difference between means	N.S.		$P < 0.01$		$P < 0.01$	

4. The nephrotic state

(a) Low density lipoproteins

A group of 13 patients with the typical clinical picture of the active nephrotic syndrome was studied. Eleven of the thirteen cases were children below the age of 10 years. There were 10 male patients ranging in age from 3 years to 41 years and 3 female patients ranging in age from 3 years to 6 years of age. No significant difference in low density lipoprotein values could be demonstrated between the nephrotic state in adults and the nephrotic state in children. The values for the four low density lipoprotein classes are presented in Table 17.

In the nephrotic state the mean levels for all four low-density lipoprotein classes are extremely elevated. This is the only metabolic disorder yet found characterized by marked elevation of all four low density lipoprotein classes.

(b) High-density lipoproteins

High density lipoprotein levels are available for 5 patients with active nephrotic syndrome (5 males, ranging from 19 to 68 years). The mean levels for the three high density lipoprotein classes are presented in Table 18.

No significant difference for HDL_1 or HDL_3 levels in the nephrotic state from those in matched controls can be demonstrated. The direction of change is toward an elevation in HDL_1 level and a depression in HDL_3 level, but, if significant, a larger series would be required to prove these changes. The HDL_2 lipoproteins are, however, markedly and significantly reduced in the nephrotic state as compared with matched controls. A comparison of the nephrotic state with xanthoma tendinosum, xanthoma tuberosum, and chronic biliary obstruction is interesting. All those states show significant reductions in HDL_2 levels, but show completely differing patterns amongst the low density lipoproteins. Thus it would be highly unlikely that a single, identical basis exists for the HDL_2 reduction in these diverse metabolic states.

5. Chronic biliary obstruction

(a) Low density lipoproteins

A group of six patients presenting the typical clinical features of chronic biliary obstruction with or without xanthomatosis was studied. The etiology of the biliary obstruction in several of these cases remained obscure in spite of

Table 21 Low Density Lipoproteins in "Essential Hyperlipemia"

	S_f°0–12 mg/100 ml	S_f°12–20 mg/100 ml	S_f°20–100 mg/100 ml	S_f°100–400 mg/100 ml
Mean lipoprotein levels in patients with essential hyperlipemia (9 cases)	229	66	450	967
Range of levels in essential hyperlipemia	130–320	18–92	184–1100	132–2937
Mean lipoprotein levels on matched controls	364	68	109	83
Difference in mean levels in standard score units	−1.6	−0.1	+7.1	+11.5
Significance test on the difference between means	$P < 0.01$	N.S.	$P \ll 0.01$	$P \ll 0.01$

Table 22 High Density Lipoproteins in Essential Hyperlipemia

	HDL_1 mg/100	S. D.	HDL_2 mg/100	S. D.	HDL_3 mg/100	S. D.
Mean HDL levels in essential hyperlipemia (5 cases)	80.0	26.0	54.0	31.4	144.2	98.0
Range of levels in essential hyperlipemia	30–102		14–71		61–300	
Mean levels on matched controls	19.5		79.9		195.6	
Difference in means in mg/100 ml (essential hyperlipemia-matched controls)	+60.5		−25.9		−51.4	
Significance test on the difference between means	$P < 0.01$		N.S.		N.S.	

investigation, including laparotomy. This group constituted one male of age 33 years and five females ranging in age from 24 to 52 years of age. The values for the four low density lipoprotein classes are presented in Table 19.

In chronic biliary obstruction massive elevations are the rule in the levels of $S_f°0–12$ and $S_f°12–20$ lipoproteins. The $S_f°20–100$ lipoprotein level may or may not be grossly elevated. The $S_f°100–400$ lipoproteins show a mean level not differing significantly from that in the matched controls. Chemical and physico-chemical studies of the low density lipoproteins in biliary obstruction to be reported elsewhere[22] show that certain of the lipoproteins in this disease differ from those in other states even though their flotation rates are similar under the conditions employed in these studies.

(b) High density lipoproteins

High density lipoproteins were studied in 5 patients with chronic biliary obstruction (1 male, age 33, 4 females ranging in age from 20 to 49 years). The mean levels for the three high density lipoprotein classes are presented in Table 20.

Of especial note is the *extremely* marked reduction in both HDL_2 and HDL_3 observed in chronic biliary obstruction. The levels are so inordinately low in this state as to justify the term, "wipe out" of lipoproteins, for these two high density classes. Consideration of the range of values encountered indicates that even for the case showing the highest HDL_2 and HDL_3 levels, there is marked reduction compared with the mean for matched controls. The HDL_1 reduction in chronic biliary obstruction cannot be proven significant within the available data.

6. "Essential Hyperlipemia"

(a) Low density lipoproteins

"Essential hyperlipemia" represents a vaguely defined clinical state, usually discovered by the incidental finding of creamy serum in a fasting blood specimen. Manifestly, the criteria for such a diagnosis can hardly be regarded as quantitative or definitive. Some authors refer to xanthoma tuberosum as "essential hyperlipemia" in those patients with the typical cutaneous tuberous lesions in whom the fasting serum is creamy. In our considerations of "essential hyperlipemia" reference is made only to those patients referred for study because of creamy serum, but who do not show any visible xanthomatous lesions. Nine such patients were available for low density lipoprotein analysis, including 7 males ranging in age from 29 to 47 years and 2 females of 50 and 68 years of age, respectively. The values for the four low density lipoprotein classes are presented in Table 21.

The data indicate "essential hyperlipemia" to be characterized by massive elevations in the $S_f°100–400$ and $S_f°20–100$ lipoprotein levels. The Sf°12–20 levels are not signif-

Table 23 The Relationship of Diastolic Blood Pressure with Low Density Lipoproteins

	n = 309 cases 40–49 year old males Pearson Product-Moment Correlation coefficient r	Significance Test	n = 446 cases 30–39 year old females Pearson Product-Moment Correlation coefficient r	Significance Test
Diastolic pressure vs $S_f°0–12$	+0.093	N.S.	+0.125	$P < 0.05$
Diastolic pressure vs $S_f°12–20$	+0.005	N.S.	+0.051	N.S.
Diastolic pressure vs $S_f°20–100$	+0.153	$P < 0.01$	+0.136	$P < 0.01$
Diastolic pressure vs $S_f°100–400$	+0.208	$P < 0.01$	+0.147	$P < 0.01$

Table 24 Relationship of Diastolic Blood Pressure with High Density Lipoproteins

	Age Group	No. of Subjects	r, Diastolic B.P. vs. HDL_1	Significance Test	r, Diastolic B.P. vs. HDL_2	Significance Test	r, Diastolic B.P. HDL_3	Significance Test
Males								
	30–39	83	+0.04	N.S.	−0.09	N.S.	−0.04	N.S.
	40–49	69	+0.09	N.S.	−0.17	N.S.	+0.07	N.S.
	50–59	53	+0.08	N.S.	−0.18	N.S.	+0.13	N.S.
Females								
	30–39	155	+0.04	N.S.	−0.15	$p = 0.05$	−0.02	N.S.
	40–49	154	+0.01	N.S.	−0.09	N.S.	−0.29	$P < 0.01$
	50–59	78	+0.16	N.S.	−0.27	$0.05 > P > 0.01$	+0.03	N.S.

icantly different from those in matched controls. The Sf°0–12 lipoproteins are quite markedly lower than those for matched controls. The general low density lipoprotein distribution is somewhat similar to that in xanthoma tuberosum. However in essential hyperlipemia the $S_f°100–400$ is in general *higher* than in xanthoma tuberosum, and both the $S_f°12–20$ and the $S_f°20–100$ is in general *lower* than in xanthoma tuberosum.

(b) High Density Lipoproteins

High density lipoprotein measurements are available for 5 patients (4 males and 1 female). The mean levels for the three high density lipoprotein classes in essential hyperlipemia are presented in Table 22.

The most striking feature of the high density lipoproteins in "essential hyperlipemia" is the massive elevation in the level of HDL_1 as compared with that in matched controls. It is of especial interest to note that in two other states with low-density lipoprotein patterns similar to that observed in "essential hyperlipemia" namely, diabetic acidosis and glycogen storage disease, this same type of marked elevation of HDL_1 occurs. It would appear that a very close relationship exists between this particular low density lipoprotein disorder and the HDL_1 elevation, independent of the disease state in which it occurs.

Although the direction of HDL_2 and HDL_3 levels is toward a reduction in comparison with those in matched controls, neither can be shown to be significantly reduced, within these data.

7. Hypertension

(a) Low density lipoproteins

Hypertension represents a disorder or group of disorders the metabolic basis of which is unknown. Since so much has been written concerning the relationship of hypertension with coronary heart disease and arteriosclerosis, it is of interest to know the extent to which factors raising blood pressure are related to the serum lipoprotein levels. In the population samples previously described,[9] individuals were not excluded from the series on the basis of blood pressure level. Hence such population samples provide groups where the relationships of blood pressures to lipoproteins can be assessed. Studies were made on one male population sample (40–49 year old) and one female sample (30–39 year old). The data for the low density lipoproteins are presented in the form of the Pearson product-moment correlation coefficient, r, with the measured levels of the various lipoprotein classes in Table 23.

For both males and females the only correlations between diastolic pressure and low density lipoprotein levels that can be demonstrated to be significant at the 1% level are the low order positive correlations of pressure with $S_f°20–100$ and $S_f°100–400$ lipoproteins. In a subsequent section it is demonstrated that overweight is also significantly and positively related to $S_f°20–100$ and $S_f°100–400$ lipoproteins. Since overweight is itself positively correlated with diastolic pressure ($r = +0.33$) the question may be raised as to the extent to which the observed diastolic pressure versus $S_f°20–100$ and $S_f°100–400$ lipoprotein correlations are independent of the overweight versus lipoprotein relationship. A multiple regression analysis indicates that the measured blood pressure versus lipoprotein correlation arises from the relationship between blood pressure and overweight, and hence that the independent relationship of blood pressure with lipoproteins is negligible.

(b) High density lipoproteins

Data are available for the Pearson product-moment correlation between diastolic blood pressure and the three high density lipoprotein classes. The population sample is chosen in a manner similar to that for the low density lipoprotein study, although a different set of subjects was actually investigated. The correlation data are presented in Table 24.

No individual category demonstrates a significant correlation between HDL_1 and diastolic blood pressure, although all of the very low values of r are in the positive direction.

Table 25 Relationship Between Relative Weight (**) and Low Density Lipoproteins

	r, RW vs S_f°0–12	Significance Test	r, RW vs S_f°12–20	Significance Test	r, RW vs S_f°20–100	Significance Test	r, RW vs S_f°100–400	Significance Test
Males (40–49 yrs) (n = 309)	+0.107	N.S.	+0.140	0.05 > P > 0.01	+0.277	P < 0.01	+0.391	P < 0.01
Females (30–39 yrs) (n = 446)	+0.112 *	0.05 > P > 0.01	+0.101	0.05 > P > 0.01	+0.208	P < 0.01	+0.244	P < 0.01

(*) There is a suggestion of a curvilinear regression of S_f°0–12 on relative weight, with a positive regression in the low relative weight range (up to RW = 1.1) and either zero or negative regression in the higher relative weight ranges. In all the other cases linear regression appeared to be present.
(**) Ideal weights for this calculation are taken from tables prepared by the Metropolitan Life Insurance Company.

For HDL_2 vs. diastolic pressure, the measured r values are all in the negative direction, but only two of the six values approach borderline significance. It would therefore appear that a probably significant negative correlation of very low order exists between the HDL_2 class and diastolic blood pressure. There does not appear to be even a consistent direction in the low order correlations measured between HDL_3 and diastolic blood pressure, although for one of the six groups a negative correlation significant at the 1% level was found. Overall, therefore, if any relationship of significance between HDL_3 and diastolic pressure exists, it is of very low order.

8. Obesity

(a) Low density lipoproteins

In an overall evaluation of the factors involved in the control of *serum* lipoprotein levels, total body fat content deserves consideration. A priori there exists no basis for assuming that any significant relationship exists. We do not have body fat measurement on the subjects studied, but have crudely assessed body fat content by measurement of the variable designated as *relative weight.* Relative weight (R.W.) is calculated as the ratio of actual weight to "ideal" weight (*) for a particular height. The measured Pearson product-moment correlations between relative weight and the four low density lipoprotein classes are given in Table 25.

The data of Table 25 indicate the existence of a significant positive, but low-order, correlation of relative weight with the low-density lipoproteins and that the magnitude of the relationship is greater for the lipoproteins of higher flotation rate than those of lower flotation rate. From studies reported elsewhere it appears likely that the relationship of specific dietary constituents with lipoprotein levels may be greater than that of relative weight itself.[23]

(b) High-density lipoproteins

The relationship of relative weight with the three high density lipoproteins is presented in Table 26.

These data indicate no significant relationship between relative weight and the HDL_1 class. There does appear to be a very low order negative correlation between HDL_2 and relative weight. Only in one group did the correlation between HDL_3 and relative weight approach borderline significance. Overall what relationships exist between high

Table 26 Relationship of Relative Weight with High-Density Lipoproteins

	Age Group	n	r, RW vs HDL_1	Significance Test	r, RW vs HDL_2	Significance Test	r, RW vs HDL_3	Significance Test
Males								
	30–39	83	−0.16	N.S.	−0.06	N.S.	−0.23	0.05 > P > 0.01
	40–49	69	+0.21	N.S.	−0.24	p = 0.05	−0.04	N.S.
	50–59	53	+0.07	N.S.	−0.30	0.05 > P > 0.01	−0.11	N.S.
Females								
	30–39	155	+0.04	N.S.	−0.16	0.05 > P > 0.01	−0.08	N.S.
	40–49	154	+0.04	N.S.	−0.02	N.S.	0.0	N.S.
	50–59	78	+0.14	N.S.	−0.23	0.05 > P > 0.01	+0.05	N.S.

Table 27 Low and High Density Lipoproteins in Ten Patients in Diabetic Acidosis

Patient No.	Age	Sex	Clinical State	S_f°0–12 mg/100 ml	S_f°12–20 mg/100 ml	S_f°20–100 mg/100 ml	S_f°100–400 mg/100 ml	HDL_1 mg/100 ml	HDL_2 mg/100 ml	HDL_3 mg/100 ml
1 (*)	37	F	Marked Acidosis No coma	195	155	1120	3739	168	29	167
2	44	F	Acidosis and coma	172	65	343	383	3	38	76
3 (*)	55	F	Acidosis No coma	235	54	1082	1622	217	19	84
4	21	M	Acidosis Semi-coma	74	9	334	865	Not analyzed		
5	11	F	Acidosis Semi-coma	0	0	416	700	77	0	0
6	21	F	Acidosis No coma	179	81	656	647	109	19	84
7	39	F	Acidosis Coma	408	139	264	291	Not analyzed		
8	35	M	Acidosis Semi-coma	401	40	20	7	30	96	203
9	52	F	Acidosis No coma	356	105	164	72	15	77	129
10	13	F	Acidosis No coma	226	94	105	31	Not analyzed		

(*) Cutaneous xanthomata were present in patients 1 and 3.

density lipoproteins and relative weight are very low order at best.

9. Diabetes Mellitus

The difficulty involved in obtaining a group of "random" diabetic subjects for study of the nature of lipid metabolic disturbances is great, especially in view of the fact that in most sources some degree of insulin (plus or minus dietary) therapy is already in use. Of special interest have been the findings encountered in diabetic patients going into or out of acidosis and/or coma, since here one has the opportunity to observe lipoprotein variation at differing stages of control of the diabetes.[17,18,24] In our experience with 10 cases of diabetes mellitus either in marked acidosis or diabetic coma, 6 cases showed a special lipoprotein disturbance characterized by a low-density lipoprotein pattern extremely similar to that observed in "essential hyperlipemia." Some of these patients have been followed as they came out of acidosis, and one case as she later became mildly acidotic again. Coma was not a prerequisite for the most severe lipoprotein alterations, since some of the patients with the most marked lipoprotein disturbances were acidotic, but not comatose. Conversely, some of the comatose patients showed minimal lipoprotein disturbance. Since there is great variation in the clinical state of the patients, no averages are made, but rather the initial blood studies in 10 patients are recorded in Table 27.

Patient Number 1 was studied serially at intervals of several days during the course of therapy of the diabetic acidosis and re-establishment of diabetic control.[17–19] The serial lipoprotein findings are presented below in Table 28.

It is to be noted that the derangement in lipoprotein transport, when present and severe is characterized by a marked elevation in S_f°20–100 and S_f°100–400 low-density lipoprotein levels and by a marked elevation in the level of the HDL_1 class of high-density lipoproteins. The S_f°0–12 lipoproteins are significantly lower than for matched controls in those cases showing the marked derangement in the S_f°20–100 and S_f°100–400 lipoprotein levels. The S_f°12–20 lipoprotein level was not strikingly altered in these cases, being low in some and high in others. The mean HDL_2 and HDL_3 lipoproteins in the seven patients in diabetic acidosis for whom such analyses had been made showed a significant ($p = 0.01$) reduction as compared with matched controls. With respect to the alterations in diabetic acidosis observed in the S_f°0–12, S_f°12–20, S_f°20–100, S_f°100–400, and HDL_1 lipoprotein classes, there is a close similarity to the findings made in "essential hyperlipemia."

The relative acuteness of the lipoprotein alterations with variation in the clinical and biochemical state of the diabetic decontrol is indeed remarkable. As is noted in Table 28 gross changes in lipoprotein distribution may occur within a period of days during the therapy of diabetic acidosis. The general shifts observed during the therapy of diabetic acidosis may be described as an

Table 28 Serial Lipoprotein Studies During the Therapy of Diabetic Acidosis

Day after Hospital Admission	Clinical State	S_f°0–12 mg/100 ml	S_f°12–20 mg/100 ml	S_f°20–100 mg/100 ml	S_f°100–400 mg/100 ml	HDL_1 mg/100 ml	HDL_2 mg/100 ml	HDL_3 mg/100 ml
0	In acidosis & coma	195	155	1120	3739	168	29	167
4 days	Out of acidosis.	444	352	1942	1530	141	7	179
9 days	In diabetic control	744	428	1277	685	66	7	179
14 days	In diabetic control	939	338	670	139	34	14	172
29 days	In diabetic control	614	134	493	228	41	33	203
43 days	In diabetic control	531	148	432	132	25	33	219
50 days	In diabetic control	616	150	332	152	19	19	192
56 days	In diabetic control Discharged from Hospital	549	108	150	31	Not analyzed		
116 days	Supposedly in control at home but showing acetonuria	452	237	988	461	52	29	157
400 days	Supposedly in control at home but showing acetonuria	276	188	968	840	Not Analyzed		

overall lowering of low-density lipoproteins levels, coupled with a shift in distribution toward lipoproteins from those of high flotation classes to those of successively lower flotation classes. During this shift the lower flotation classes, such as S_f°0–12 rises from a lower-than-average value to quite elevated levels. The HDL_1 lipoprotein level, inordinately elevated in diabetic acidosis shows a progressive fall toward average values for controls during the course of therapy. No significant shifts in HDL_2 and HDL_3 lipoproteins were detectable during the course of therapy, suggesting that the lowered levels observed in these diabetics characterizes their disease rather than the acute episode of diabetic decontrol.

Also shown in Table 28 for the patient followed serially is the sensitivity of the lipoprotein system to the state of diabetic control. Once discharged from the hospital this patient did not maintain as careful control of her diabetes as had been possible under hospital conditions, and as a result she again showed acetonuria although there was no clinical evidence of acidosis. The lipoprotein levels during this period of acetonuria showed a reversion toward the levels characteristic of the intermediary period during the therapy of diabetic acidosis.

Two of the 10 patients showed cutaneous "eruptive" xanthomata in a distribution similar to that observed in patients with chronic xanthoma tuberosum. These two patients (1 and 3) showed the highest S_f°20–100 and S_f°100–400 levels out of the entire group. The levels of these lipoprotein classes are entirely comparable with those observed in the patients with xanthoma tuberosum. It appears most reasonable to consider the eruptive xanthomatosis of diabetic acidosis to have the same basis, namely elevation of S_f°20–100 and S_f°100–400 lipoprotein levels, as does xanthoma tuberosum. The "eruptive" character of the diabetic xanthomatosis is most probably related to the fact that the lipoprotein level elevation is a relatively acute process associated with the acidosis, rather than a chronic one.

Table 29 The Serum Lipoproteins in Two Adult Cases of Glycogen Storage Disease

Patient	Sex	Age	S_f°0–12 mg/100 ml	S_f°12–20 mg/100 ml	S_f°20–100 mg/100 ml	S_f°100–400 mg/100 ml	HDL_1 mg/100 ml	HDL_2 mg/100 ml	HDL_3 mg/100 ml
1	M	27 yrs.	320	69	623	934	82	0	93
2	M	33 yrs.	703	293	1414	2151	225	0	140
Average for matched controls			321	57	94	58	19.6	59	182

Table 30 Lipoprotein Findings in Two Cases of Spontaneous Myxedema

Case	Age	Sex	S_f°0–12 mg/100 ml	S_f°12–20 mg/100 ml	S_f°20–100 mg/100 ml	S_f°100–400 mg/100 ml	HDL_1 mg/100 ml	HDL_2 mg/100 ml	HDL_3 mg/100 ml
1	44	F	730	130	112	18	Not Analyzed		
2	60	F	827	193	103	16	34	53	153

10. Glycogen storage disease

Glycogen storage disease is a disorder characterized by impaired carbohydrate metabolism and fasting hypoglycemia, and often associated with hypercholesteremia and hyperlipemia. Kolb and some of the present authors have had the opportunity to study two adult cases of glycogen storage disease, both of whom also showed hyperuricemia and gout.[18] The low and high density lipoprotein findings are presented in Table 29.

In almost every respect, the lipoprotein distribution in these two cases is remarkably similar, and grossly different from the average for matched controls, the only exception being that there was a considerable S_f°0–12 and S_f°12–20 lipoprotein elevation in one case but not in the other. For the S_f°20–100, S_f°100–400, HDL_1, HDL_2, and HDL_3 lipoproteins the findings resemble strongly those seen in the severely deranged cases of diabetic acidosis. Also, as has been pointed out previously both glycogen storage disease and diabetic acidosis show lipoprotein derangements simulating closely those noted in "essential hyperlipemia." Kolb[18] has suggested that the lipoprotein derangement common to diabetic acidosis and glycogen storage disease may have as an underlying basis the impaired carbohydrate utilization in spite of the fact that one disorder is characterized by fasting hyperglycemia and the other by fasting hypoglycemia. The question arises, from the lipoprotein similarity between "essential hyperlipemia" and diabetic acidosis or glycogen storage disease, whether there may exist a carbohydrate metabolic derangement of some type in "essential hyperlipemia" as well. No overt disorder of carbohydrate metabolism has been reported in "essential hyperlipemia," but certainly a careful search for one appears warranted.

11. The thyroid gland and lipoprotein metabolism

Two cases of spontaneous myxedema in adults have been observed before the institution of thyroid therapy. The low and high density lipoproteins findings in these two cases are given in Table 30.

It is evident even from the small series of two cases that there is a marked elevation in S_f°0–12 and S_f°12–20 in spontaneous myxedema. The S_f°20–100 and S_f°100–400 lipoproteins are not elevated. These studies plus the well-established older knowledge of the lowered serum cholesterol level in hyperthyroidism and the elevated serum cho-

Table 31 Study of 11 Cases on 650 Mg. of Thyroid Substance for 9 Weeks (*)

Weeks of Study	Mean S_f°0–12 mg/100 ml	Mean S_f°12–20 mg/100 ml	Mean S_f°20–100 mg/100 ml	Mean S_f°100–400 mg/100 ml
Control Period	343	86	112	65
1	279	40	78	40
3	225	38	82	33
4	199	35	79	31
6	237	39	75	27
7	231	44	88	36
9	256	43	85	34
11	242	38	70	29
Off Thyroid				
12	324	51	92	41
13	416	71	82	43
15	399	62	79	48
16	401	93	81	35
18	430	76	76	27
20	385	69	87	35

(*) Dosage schedule was 195 milligrams for 7 days then 390 milligrams for 6 days and then 650 milligrams for the remaining 63 days during which thyroid was given.

Table 32 Study of 27 Cases on 195 mg/day of Thyroid Substance for 33 Weeks

Weeks on 195 mg/day of thyroid	Dosage of thyroid substance in mg/day	Mean S_f°0–12 (mg/100 ml)	Mean S_f°12–20 (mg/100 ml)	Mean S_f°20–100 (mg/100 ml)	Mean S_f°100–400 (mg/100 ml)
0	0	358.8	73.1	81.8	38.0
3	195	242.9	42.5	84.0	35.0
6	195	281.4	42.6	91.0	38.4
9	195	288.7	47.4	96.5	45.9
12	195	302.0	48.5	76.2	27.3
15	195	307.4	42.1	87.6	29.5
18	195	—	—	—	—
21	195	338.4	40.6	84.7	33.9
24	195	374.6	33.9	81.2	26.7
27	195	348.9	54.1	84.5	36.0
30	195	—	—	—	—
33	195	338.3	50.6	85.7	37.4

lesterol level in hypothyroidism and myxedema bring to the fore the question of the extent to which thyroid function, even in ostensibly euthyroid individuals may be involved in the control especially of S_f°0–12 and S_f°12–20 levels in the population at large. Several approaches to this facet of lipoprotein metabolism are currently under investigation. One such approach involves the study of response to various dosage levels of exogenous thyroid substance in groups of euthyroid individuals.[25,26] The earliest studies were carried out utilizing the large dose of 650 milligrams of desiccated thyroid substance per day in 11 patients, who were ostensibly euthyroid. The average results of 9 weeks of administration of 650 milligrams of thyroid substance per day to 11 patients is shown in Table 31.

A study of individual cases in this group, in addition to the study of mean values, revealed that uniformly S_f°0–12 and S_f°12–20 lipoproteins showed a pronounced fall during the 9 week period of administration of 650 milligrams per day of thyroid substance. Some patients did show drops in S_f°20–100 and S_f°100–400 lipoproteins, but this was not so in all cases. It is interesting, therefore, that the administration of exogenous thyroid substance produced lipoprotein alterations for the same flotation classes, but in the opposite direction to the findings observed in spontaneous myxedema as contrasted with matched controls. It is evident that exogenous thyroid extract can markedly influence S_f°0–12 and S_f°12–20 lipoprotein levels in all the cases studied with the large dose employed. Investigation was then directed toward determination of how low the exogenous thyroid substance dose could be and still effect an alteration in lipoprotein levels. Preliminary studies had indicated that doses of the order of 65 to 130 milligrams per day were ineffective.[27] Hence a study was initiated at a dosage level of 195 milligrams per day of desiccated thyroid substance. The average results for 27 cases in a 33 week period of administration of thyroid are given in Table 32.

From the data of Table 32 it is evident that a marked lipoprotein lowering was achieved in the 3 to 6 weeks period following the institution of thyroid administration, but that thereafter levels rose in spite of continuation of the thyroid. It was found that at three weeks of administration the magnitude of the fall in S_f°0–20 lipoprotein level was inversely related to the initial S_f°0–20 lipoprotein level. These data are presented in Table 33.

It would appear reasonable to consider that one possible explanation for the observations is that the group of individuals with the highest S_f°0–20 levels are on the average, manufacturing and utilizing the least endogenous thyroid substance per

Table 33 Relationship of Maximal Lowering (*) of S_f°0–20 Lipoproteins to Pre-Thyroid Level of S_f°0–20 Lipoproteins

Range of Pre-Thyroid S_f°0–20 Lipoprotein Concentration (mg/100 ml)	Number of Cases	Mean Pre-Thyroid S_f°0–20 Lipoprotein concentration (mg/100 ml)	Mean S_f°0–20 Lipoprotein at 3 wks. on 195 milligrams of thyroid daily (mg/100 ml)	Mean Change in S_f°0–20 Lipoprotein concentration (mg/100 ml)
Over 500 mg/100 ml	15	556	366	190
400–499 mg/100 ml	22	453	310	143
300–399 mg/100 ml	12	363	250	113
Under 300 mg/100 ml	9	248	207	41

(*) Maximal Lowering in S_f°0–20 lipoprotein concentration is observed, within these data, to occur at 3 weeks after the institution of 195 milligrams of thyroid substance daily.

Table 34 The Serum Lipoproteins in Pregnancy and in the Post-Partum State

	2nd trimester (7 cases)	3rd trimester (9 cases)	Delivery to 5 days post-partum (9 cases)	5 days to 6 weeks post-partum (7 cases)	9 months post-partum (3 cases)
S_f°0–12 (MG/100 ML)					
Mean for pregnant women	309.7	369.2	348.3	381.7	269.3
Mean for matched controls	298.6	298.6	298.6	298.6	298.6
Difference	+11.1	+70.6	+49.7	+83.1	−29.3
Significance test on difference	N.S.	0.05 > P > 0.01	0.05 > P > 0.01	0.05 > P > 0.01	N.S.
S_f°12–20 (MG/100 ML)					
Mean for pregnant women	54.6	105.2	76.8	65.7	34.7
Mean for matched controls	47.7	47.7	47.7	47.7	47.7
Difference	+6.9	+57.5	+29.1	+18.0	−13.0
Significance test on difference	N.S.	0.05 > P > 0.01	0.05 > *P* > 0.01	0.05 > P > 0.01	N.S.
S_f°20–100 (MG/100 ML)					
Mean for pregnant women	63.4	141.3	126.1	77.1	67.3
Mean for matched controls	56.4	56.4	56.4	56.4	56.4
Difference	+7.0	+84.9	+69.7	+20.7	+10.9
Significance test on difference	N.S.	0.05 > P > 0.01	0.05 > P > 0.01	0.05 > P > 0.01	N.S.
S_f°100–400 (MG/100 ML)					
Mean for pregnant women	15.9	34.2	41.8	31.6	24.7
Mean for matched controls	20.9	20.9	20.9	20.9	20.9
Difference	−5.0	+13.3	+20.9	+10.7	+3.8
Significance test on difference	N.S.	N.S.	0.05 > P > 0.01	N.S.	N.S.
HDL_1 (MG/100 ML)					
Mean for pregnant women	20.4	21.7	23.8	22.0	17.0
Mean for matched controls	15.1	15.1	15.1	15.1	15.1
Difference	+5.3	+6.6	+8.7	+6.9	+1.9
Significance test on difference	0.02 > P > 0.01	0.05 > P > 0.01	P < 0.01	0.05 > P > 0.01	N.S.
HDL_2 (MG/100 ML)					
Mean for pregnant women	168.3	151.0	117.8	94.3	52.2
Mean for matched controls	121.0	121.0	121.0	121.0	121.0
Difference	+47.3	+30.0	−3.2	−26.7	−69.8
Significance test on difference	0.02 > P > 0.01	N.S.	N.S.	N.S.	N.S.
HDL_3 (MG/100 ML)					
Mean for pregnant women	240.0	254.7	257.6	282.4	220.5
Mean for matched controls	207.0	207.0	207.0	207.0	207.0
Difference	+33.0	+47.7	+50.6	+75.4	+12.5
Significance test on difference	0.05 > P > 0.01	P = 0.01	0.02 > P > 0.01	P < 0.01	N.S.

day and hence the addition of 195 milligrams per day provides the greatest percentage increment in available thyroid. The explanation we consider most likely for the gradual return of the lipoprotein levels to nearly the pre-thyroid administration values lies in the interrelationship between the thyroid and pituitary gland. Thyrotropin secretion by the pituitary is considered to be suppressed by increased circulating levels of the thyroid hormone itself. Thus when 195 milligrams of exogenous thyroid substance are given per day, the early effect is to increase the circulating level of thyroid hormone. This is accompanied by S_f°0–12 and S_f°12–20 lipoprotein reduction. However at the same time thyrotropin secretion is suppressed, and secondary to this, endogenous thyroid secretion is suppressed. If this suppression of thyroid secretion were equivalent to 195 milligrams per day, the *net* available thyroid substance for metabolism is the same as it was at the outset, before administration of thyroid, and the observed return of lipoprotein levels to those prevailing at the outset would be anticipated. If this concept is correct, the successive increase of dosage of exogenous thyroid substance should finally reach a point where even with *complete* suppression of endogenous thyroid production there will be more available thyroid hor-

Table 35 Serum Lipoprotein Levels in Three Cases of Umbilical Cord Blood

Case	S_f°0–12 mg/100 ml	S_f°12–20 mg/100 ml	S_f°20–100 mg/100 ml	S_f°100–400 mg/100 ml	HDL_1 mg/100 ml	HDL_2 mg/100 ml	HDL_3 mg/100 ml
1	67	16	31	0	9	19	115
2	0	0	0	0	9	0	51
3	58	20	16	2	Not Analyzed		

mone than at the outset and lipoprotein levels should remain lowered. The experience with 650 milligrams per day is consistent with this concept, and present studies with the dosage elevated to 260 and then 325 grains per day, following a period on 195 milligrams while not yet completed, also appear consistent with this concept.

12. The state of pregnancy

The striking difference in the aging trends in lipoprotein distributions observed between the ostensibly healthy male and female human suggests that a search in the direction of the gonadal hormones might provide some clues as to the basis for the observed male-female lipoprotein differences. One approach to this, among others being made in our laboratory, has involved a study of the serial lipoprotein findings during and after a pregnancy in the female, since major hormonal alterations occur during such periods.[20] Such studies have been made in nine females followed through some or all of the period of gestation and the post-partum period of an uncomplicated pregnancy. The lipoprotein results are presented in Table 34. It is apparent from these data that significant lipoprotein alterations do occur, both in the low and high density lipoprotein classes, during pregnancy and the post-partum period thereafter. Further, the various lipoprotein classes appear to show differing trends, both in the direction of lipoprotein level alteration and in the chronology of the alterations. It appears worthwhile to extend such studies and to interrelate them with hormonal alterations in the effort to understand the underlying mechanisms responsible for the observed changes.

Of additional interest are the lipoprotein findings in the umbilical cord blood of the infants from several of these pregnancies. The data are presented in Table 35. It is evident from these data that the lipoproteins do not equilibrate between maternal and infant blood, since for several lipoprotein classes where the maternal blood shows high levels, the infant blood from the umbilical cord shows extremely low lipoprotein levels.

13. Heparinemia

There may be a question concerning the inclusion of "heparinemia" among metabolic states associated with lipoprotein alterations. However, observations of the effect of parenteral heparin injection are so striking and of such potential importance with respect to physiological lipoprotein transformations as to necessitate their discussion here. In 1943 Hahn[28] made the very important observation that the turbidity in the serum of dogs post-prandially could be reduced by the parenteral administration of heparin. He

Table 36 The Chemical Distribution of Lipids Among the Lipoproteins Before and After Injection of Heparin

	Cholesterol Ester		Unesterified Cholesterol		Glycerides		Unesterified Fatty Acid		Phospho-lipid	
	Heparin		Heparin		Heparin		Heparin		Heparin	
Lipoprotein Class (*)	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
S_f°20–400	35.0	15.0	28.3	16.7	176.8	26.7	6.7	5.0	76.6	20.0
S_f°0–20	143.2	145.0	38.4	56.6	55.0	43.3	6.7	35.0	70.0	76.6
Major High-Density lipoproteins	26.7	31.6	25.0	23.4	36.7	26.7	18.3	35.0	88.3	95.0
Total Ultracentrifugal lipoprotein "residue"	7.0	20.0	3.3	10.0	3.3	16.7	6.6	56.6	—**	—**
Total	211.9	211.6	95.0	106.7	271.8	113.4	38.3	131.6		

(*) All chemical values in this table represent the number of milligrams of lipid constituent in that quantity of each lipoprotein class to be found in 100 milliliters of serum (or plasma).

(**) Phospholipid analyses are not available for this ultracentrifugal fraction.

correctly attributed the decreased turbidity (or "optical clearing") to an effect of the heparin injection upon the serum lipemia. This observation was soon extended to the lipemic turbidity of human serum. Anderson and Fawcett[29] in 1950 made another major step forward in showing that plasma withdrawn from a heparinized animal would, upon mixing *in vitro,* cause a diminution in the turbidity of a lipemic plasma. Heparin, however, added directly *in vitro* to lipemic plasma was ineffective in reduction of its turbidity. Anderson and Fawcett referred to the factor in post-heparin plasma responsible for its effect in optical "clearing" of turbidity as "anti-chylomicronemic factor." The turbidity of serum or plasma deserves consideration. For the effective scattering of light, which is the same as the production of turbidity, molecules suspended in an aqueous medium are the more effective the larger their size when present at equivalent concentrations, if concentration is expressed on a mass per unit volume basis. The ordinary serum proteins, such as albumin or gamma globulin, contribute relatively insignificantly to the turbidity of serum because of their small molecular size (molecular weights of the order of 10^5 units). The lipoproteins of serum present an entirely different situation. For the lipoprotein "spectrum" there is a very great range of molecular sizes, with molecular weights ranging from 10^5 all the way to 10^{11} units. Practically all of the serum turbidity is the result of the presence in serum of lipoproteins of the molecular weight range from 10^6 to 10^{11} (approximately $S_f°2$ to $S_f°40{,}000$). Milligram for milligram, in a given volume of serum, lipoproteins of successively higher molecular weight (and successively higher flotation rate) contribute successively greater increments to the turbidity of serum. Indeed low concentrations of lipoproteins of the chylomicron class ($S_f°$ approximately 40,000) can contribute practically all of the serum turbidity. If chylomicrons are removed from serum, the residual turbidity is produced by lipoproteins of somewhat lower molecular weights (and lower flotation rates). Since the turbidity of serum is related to the presence of one or several of the above-described lipoprotein classes, it became of prime interest to know what the relationship of the heparin-induced turbidity reduction was to the lipoproteins of serum. Graham and others[30] investigated this phenomenon and showed that parenteral injection of heparin is followed in the human by profound effects upon a large segment of the entire serum lipoprotein "spectrum". The typical change in the lipoprotein "spectrum" occurring *in vivo* following parenteral heparin injection is the lowering or actual elimination of lipoproteins of the $S_f°20-40{,}000$ class. At the same time there are increases in concentration of lipoproteins of the $S_f°0-20$ class, although not compensatory on a concentration (or weight) basis for the loss of molecules of flotation rates higher than $S_f°20$. These events suggest that the lipoproteins of high flotation rates are converted *in vivo* to those of lower flotation rates. The shift in lipoprotein distribution occurs within a matter of minutes following administration of heparin and persists over a period of hours. Subsequently there follows a gradual return, generally

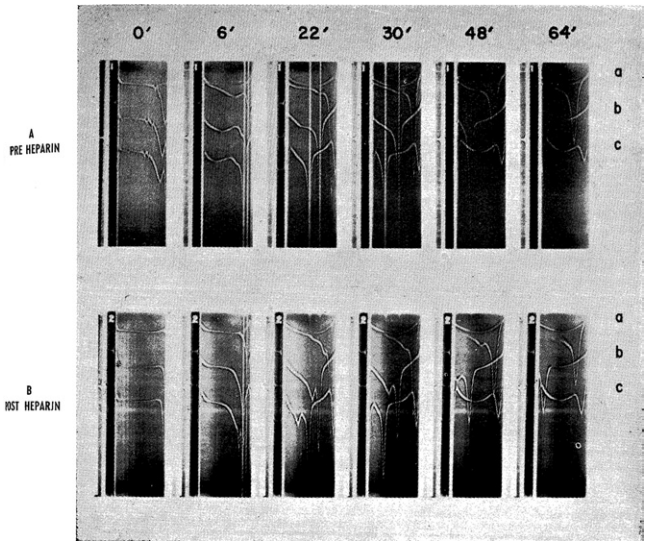

Figure 3 The ultracentrifugal lipoprotein photographs before (Picture A) and 10 minutes after (Picture B) the intravenous injection of 10000 units of heparin sodium solution.

within 24–48 hours, to the original pre-heparin lipoprotein distribution. Lindgren, Nichols and Freeman[11] have studied in detail the changes in chemical composition of the lipoprotein classes accompanying the lipoprotein redistribution which results *in vivo* following parenteral heparin administration. In that study serum was ultracentrifugally fractionated in the same manner as that described in section II on the chemical composition of the serum lipoproteins. The data in Table 36 present the alterations in chemical distribution of lipids among the lipoproteins obtained before and 10 minutes after the intravenous injection of 10,000 units of sodium heparin in a fasting individual characterized by appreciable serum levels of major high density lipoproteins, $S_f°0-20$, and $S_f°20-400$ lipoproteins (see Figure 3). The major significant features observed are as follows:

Each picture shows 3 separate analytical ultracentrifugal runs.

(a) Upper pattern in all frames of both pictures represents ultracentrifugal flotation run at 1.063 gms/ml.
(b) Middle pattern in all frames of both pictures represents ultracentrifugal flotation run at 1.125 gms/ml.
(c) Lower pattern in all frames of both pictures represents ultracentrifugal flotation run at 1.20 gms/ml.

All runs are made at 52,640 RPM, 26°C, distance from center of rotation to cell base = 72.5 mm., with photographic frames taken at 0, 6, 22, 30, 48, and 64 minutes after the rotor has reached full speed. (See references 3 and 8 for further details of interpretation).

Ten minutes after heparin injection:

(1) There is a 58% drop in *total* glyceride content of the four ultracentrifugal fractions combined.
(2) There is approximately a 3 1/2-fold increase in the total content of unesterified fatty acids. This rise brings the

Table 37 The Chemical Composition of Serum Lipoproteins Before and After Heparin Injection

Lipoprotein Class	Cholesterol Ester	Unesterified Cholesterol	Glycerides	Unesterified Fatty Acidis	Phospholipid
S_f°20–400					
Preheparin	10.8%	8.8%	54.6%	2.1%	23.7%
Postheparin	18.0%	20.0%	32.0%	6.0%	24.0%
S_f°0–20					
Preheparin	45.7%	12.2%	17.5%	2.1%	22.4%
Postheparin	40.6%	15.9%	12.1%	9.8%	21.5%
Major High Density Lipoproteins					
Preheparin	13.7%	12.8%	18.8%	9.4%	45.3%
Portheparin	15.0%	11.0%	12.6%	16.5%	44.9%

total unesterified fatty acid content much above physiologic levels usually encountered.

(3) There is no significant change in the *total* content either of cholesterol ester or of unesterified cholesterol.

(4) In the S_f°20–400 class there is a marked loss of all chemical constituents with the exception of unesterified fatty acids. The glyceride loss is the most marked.

(5) In the S_f°0–20 class there is a marked rise in unesterified fatty acid content, a lesser rise of unesterified cholesterol, and a fall of borderline significance in glyceride content.

(6) In the major high density lipoproteins there is a marked rise in unesterified fatty acid content and a minimal fall in glyceride content.

(7) In the ultracentrifugal lipoprotein "residue" all components analyzed showed increases, especially marked being the rise in unesterified fatty acids and in glyceride.

These findings are best interpreted after consideration of the changes in percentage composition of lipids in the lipoproteins, which changes are presented in Table 37.

The marked loss from the serum of glyceride during the period following intravenous heparin injection is uniformly observed in individuals possessing appreciable S_f°20–400 lipoproteins, which are being transformed. The *in vivo* experiments alone leave open the possibility that the glyceride lost may either have become attached to cellular elements of the blood or may have left the blood compartment as glyceride.

That neither of such possibilities is the correct explanation has been shown by *in vitro* incubations of S_f°20–400 lipoproteins with plasma or plasma fractions from a heparinized individual. In such *in vitro* experiments, glyceride shows a marked decrease just as it does in the above *in vivo* experiment. Shore, Nichols, and Freeman[31] have resolved this problem by demonstrating that the injection of heparin results in the activation of an enzyme which hydrolyzes glycerides to produce free fatty acids. This hydrolysis is the basis for the loss in glyceride content both *in vivo* and *in vitro*. This is the basis for marked drop in glyceride content of S_f°20–400 lipoproteins noted in Table 36. The unesterified fatty acids produced by the heparin-activated hydrolysis of glycerides have become distributed largely to three ultracentrifugal fractions, namely to the S_f°0–20 class, the major high density lipoprotein class, and to the ultracentrifugal lipoprotein "residue". This accounts for the rise of total unesterified fatty acid content of these three ultracentrifugal fractions noted in Table 36. Shore and coworkers[31] showed further that neither phospholipid nor cholesterol ester serves as a substrate for the heparin-activated lipolytic enzyme. This is consistent with the observations in Table 36 which indicate practically no change in total cholesterol ester content of the lipoproteins following heparin injection. It does appear, however, from those data that in the course of heparin-induced transformation of a large quantity of S_f°20–400 lipoprotein, there is a shift of some cholesterol ester from S_f°20–400 to the ultracentrifugal lipoprotein "residue." Similarly, unesterified cholesterol, which would not be anticipated to be affected by the heparin-activated lipolytic enzyme shows no change in total serum content (see Table 36). Here again, however, there appears to have been a shift of some unesterified cholesterol from the transformed S_f°20–400 lipoproteins into the ultracentrifugal lipoprotein "residue" and some into the S_f°0–20 class.

The lipoprotein chemical composition changes accompanying the lipoprotein transformation and glyceryl ester hydrolysis can be considered from the data presented in Table 37. Much of the S_f°20–400 lipoprotein originally present was transformed in the 10 minutes after heparin injection. What residual S_f°20–400 lipoproteins were present were highly weighted toward the S_f°20 region, whereas the original S_f°20–400 lipoproteins were distributed throughout the S_f°20–400 region. Thus the shift from 10.8% cholesterol ester in pre-heparin S_f°20–400 lipoproteins to 18.0% is consistent with the chemical data of Section II which indicated that lipoproteins of flotation rate near S_f°20 are normally much higher in cholesterol ester than those of higher flotation rates. The fall in glyceride percentage from 54.6% in pre-heparin S_f°20–400 to 32.0% in post-heparin S_f°20–400 is also consistent with the chemical data of Section II. The relatively high percentage of unesterified cholesterol in the post-heparin residual S_f°20– 400 lipoproteins is greater

than would be expected from the chemical data of Section II for such lipoproteins. Lipoproteins of flotation rates well above $S_f°20$ have more unesterified cholesterol in relationship to the cholesterol ester content than do lipoproteins of flotation rate near $S_f°20$. It would therefore appear that the post-heparin residual $S_f°20-400$ lipoproteins have attached some of the excess available unesterified cholesterol, thus accounting for the relatively high percentage of this constituent in the post-heparin $S_f°20-400$ of Table 37. The phospholipid percentage composition of pre-heparin $S_f°20-400$ is not significantly different from that of post-heparin $S_f°20-400$, which is consistent with the chemical data of Section II. The $S_f°0-20$ lipoproteins post-heparin are shifted in distribution toward $S_f°20$ from lower flotation rates because of the influx of lipoprotein into this class as a result of the transformation of part of the $S_f°20-400$ lipoproteins into $S_f°0-20$, especially into the $S_f°10-20$ region of this class. The slight fall in cholesterol ester percentage and the rise in unesterified cholesterol percentage observed in Table 37 for post-heparin $S_f°0-20$ are therefore consistent with the chemical composition data for such lipoproteins in Section II. The absence of significant change in percentage composition of phospholipid in pre- versus post-heparin $S_f°0-20$ lipoproteins, as noted in Table 37, is consistent with the chemical data of Section II for such lipoproteins. For the major high density lipoproteins the data of Table 37 reveal no appreciable changes in percentage composition with respect to cholesterol ester, unesterified cholesterol, or phospholipid, suggesting that no drastic alterations in these constituents of the high density lipoproteins has occurred as a result of the action of the heparin-activated lipoprotein transforming system. The slight fall in glyceride percentage of these lipoproteins, if significant, may suggest that some of this glyceride can serve as a substrate for the heparin-activated enzyme.

Analysis of the ultracentrifugal diagrams (see Figure 3) and the chemical distribution data of Table 36 fail to reveal any evidence for the production *de novo* of any of the major high-density lipoproteins (HDL_1, HDL_2, or HDL_3) under the influence of heparin injection. What little increase is observed ultracentrifugally in high density lipoprotein content post-heparin is explainable by the chemically observed increment of unesterified fatty acid in this lipoprotein group. The high-density lipoprotein group contains those lipoproteins analyzed by other methods as "alpha" lipoproteins. Several workers have referred to the production of "alpha" lipoproteins from "beta" lipoproteins under the influence of parenteral heparin.[32–34] We consider this conclusion to be erroneous, since the ultracentrifugal studies and chemical data provide no evidence favoring a *de novo* production of high density ("alpha") lipoproteins from low density ("beta") lipoproteins. It is of interest to note that in general the workers report the production of "alpha" lipoproteins from "beta" lipoproteins base this conclusion upon electrophoretic studies. As is noted in Tables 36 and 37, there is an increment in percentage composition of unesterified fatty acids (from glyceride hydrolysis) in both the $S_f°20-400$ and $Sf^0 0-20$ lipoproteins after heparin action. Such an occurrence would be anticipated to provide a net increase in negative charge at the pH values usually used in electrophoretic studies and hence could result in more rapid migration electrophoretically of $S_f°20-400$ and $S_f°0-20$ lipoproteins, leading to the false impression that they represent "alpha" lipoproteins (*). Indeed the attachment of fatty acid anions to any protein or lipoprotein might be anticipated to increase electrophoretic mobility.

It is of great interest to note the significant accumulation of such lipid as cholesterol ester, unesterified cholesterol, glyceride, and unesterified fatty acid in the ultracentrifugal lipoprotein "residue." Although the phospholipid fraction was lost to analysis, there is reason to suspect that phospholipid also may have increased in the ultracentrifugal lipoprotein "residue." For the four lipid constituents analyzed, approximately one-fifth of the total serum lipids after heparin action are to be found in the ultracentrifugal lipoprotein "residue." Expressed otherwise this represents the unusual situation where one-fifth of the serum lipids are to be found elsewhere than in the usual lipoprotein "spectrum." The physicochemical form of these lipid constituents is at present not known, although there is good reason to believe[35,36] that a large part of the unesterified fatty acids in the "residue" is associated chemically with albumin. Whether or not the remaining lipid constituents of the "residue" are in some way bound to proteins in the form of sub-structural units involved in synthesis of the usual lipoproteins remains a question for future resolution.

In the considerations above attention was focused largely upon the *in vivo* effects of heparin injection upon serum lipoproteins. The concept of transformation of such lipoproteins as $S_f°20-400$ into $S_f°0-20$ lipoproteins has been directly verified by *in vitro* studies of Lindgren[37] reported here.

Since post-heparin plasma contains some low-density lipoproteins of the $S_f°0-20$ and $S_f°20-400$ classes, it is advantageous to remove all such lipoproteins from such plasma before utilizing the heparin activity in such plasma for transformation studies. With these $S_f°0-20$ and $S_f°20-400$ lipoproteins removed, it becomes possible to study the net ultracentrifugal changes resulting from the interaction of the active constituents of post-heparin plasma with such a substrate as isolated $S_f°20-400$ lipoproteins. High salt concentrations destroy the activity of post-heparin plasma. Hence to allow ultracentrifugal removal of the $S_f°0-400$ lipoprotein, deuterium oxide (D_2O) made up to 0.9 % NaCl was used to raise the solution density to 1.063 gms/ml (see Section I). The plasma so treated is then ultracentrifuged at 104,000 × G for 24 hours. All detectable lipoprotein-transforming activity of the original post-heparin plasma is contained in the bottom 2 milliliters (out of a total preparative

*It is of interest to note that after heparin action the percentage composition of unesterified fatty acid in $S_f°0-20$ and $S_f°20-400$ lipoproteins is increased to the same order of magnitude as the similar pre-heparin value for the major high density lipoproteins.

tube content of 6 milliliters), which is hereafter referred to as the *post-heparin active fraction.* In addition to all the serum proteins, there is also present quantitatively in this active fraction the two high-density lipoproteins, HDL_2 and HDL_3, as well as part of the HDL_1 lipoproteins. An isolation of $S_f°20-400$ lipoproteins, free of other lipoproteins and proteins, was made from a non-heparinized subject by the preparative centrifugal techniques described in Section II[3]. This preparation served as the substrate for transformation studies. The following types of *in vitro* incubations at 37°C for 2 hours were performed (*).

(a) Incubation of isolated $S_f°20-400$ lipoproteins alone.
(b) Incubation of isolated $S_f°20-400$ lipoproteins mixed with the above-described bottom fraction obtained from *pre*-heparin serum.
(c) Incubation of isolated $S_f°20-400$ lipoproteins mixed with the above described bottom fraction obtained from *post*-heparin plasma.

The quantitative relationships were such that the $S_f°20-400$ lipoprotein content of one milliliter of serum was incubated with the "active fraction" present in one milliliter of post-heparin plasma (or one ml. of the control fraction from pre-heparin serum). Following incubation, the low density and high density lipoproteins are isolated successively and separately by successive preparative ultracentrifugations at 1.063 gms/ml. and 1.24 gms/ml. These separate fractions were then subjected to analytical ultracentrifugation. The analytical ultracentrifugal photographs for all incubations are presented in Figure 4. In order to compare readily the fate of the $S_f°20-400$ substrate in the various incubations, tracings of Frame 2 and 3 of the low-density analytical runs are superimposed in Figure 5.

It is seen from inspection of Figures 4 and 5 that the bottom fraction from pre-heparin serum has failed to influence the ultracentrifugal distribution of the $S_f°20-400$ substrate. In marked contrast the post-heparin "active fraction" has effected an essentially quantitative transformation of the $S_f°20-400$ substrate into lipoproteins of flotation rates of $S_f°0-20$.

This directly observed *in vitro* transformation resembles strikingly the alteration in lipoproteins of this group observed *in vivo* (see Figure 3).

With respect to high density lipoproteins, Figure 4 shows there has been no detectable alteration in HDL_1 lipoprotein nor any production of unusual high-density lipoproteins. There is no detectable effect of incubation of pre-heparin bottom fraction with $S_f°20-400$ upon the HDL_2 and HDL_3 high density lipoproteins. There appears to be an increase of approximately 10% in the concentration of (HDL_2 + HDL_3) lipoproteins as a result of the incubation of $S_f°20-400$ substrate with post-heparin "active fraction". From considerations earlier in this section on *in vivo* studies, it is antic-

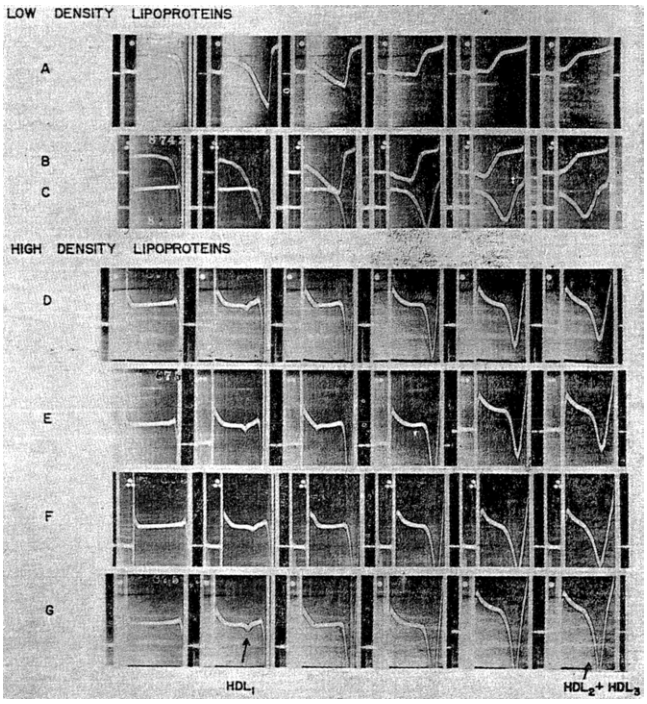

Figure 4 Ultracentrifugal lipoprotein photographs for the incubation of isolated $S_f°20-400$ lipoproteins with bottom fraction from pre-heparin serum and post-heparin "active" fraction.

Pictures A, B, and C are low density ultracentrifuge runs (density = 1.063 gms/ml) for the study of the original $S_f°20-400$ lipoproteins and the low-density transformation products.

Pictures D, E, F, and G are high-density ultracentrifuge runs (density = 1.25 gms/ml) for the study of high density lipoproteins in the "active fraction" before and after incubation.

All runs made at 52640 at 26°C, (center of rotor to cell base = 72.5 mm). In Pictures A, B, and C, frames are taken at 2 minute intervals after full rotor speed. In pictures D, E, F, and G, frames are taken at 16 minute intervals after full rotor speed.

In picture A is noted the original isolated $S_f°20-400$ lipoproteins.

In picture B is noted the pattern obtained after incubation of $S_f°20-400$ substrate with pre-heparin bottom fraction. Note the absence of any significant alterations in the $S_f°20-400$ lipoproteins.

In picture C is noted the pattern obtained after incubation of $S_f°20-400$ substrate with post-heparin "active" fraction. Note the marked decrease in $S_f°20-400$ lipoproteins as compared with Pictures A and B, and the appearance de novo of $S_f°0-20$ lipoproteins.

In picture D is noted the high density lipoprotein pattern for the HDL_1, HDL_2, and HDL_3 lipoproteins of the botton fraction of pre-heparin serum.

In picture E is noted the high density lipoprotein pattern for HDL_1, HDL_2, and HDL_3 lipoproteins of the "active" bottom fraction of post-heparin plasma.

In picture F is noted the high density lipoprotein pattern after incubation of $S_f°20-400$ substrate with pre-heparin bottom fraction. Note absence of any significant change as compared with Picture D.

In picture G is noted the high density lipoprotein pattern after incubation of $S_f°20-400$ substrate with post-heparin "active" fraction. Note that the only change in high density lipoprotein pattern is a slight increase (approximately 10%) in area (and hence concentration of the HDL_2 plus HDL_3 group of lipoproteins). (From text this is probably due to attachment of liberated fatty acid to these lipoproteins).

*Incubations were actually performed in 6 ml preparative centrifuge tubes to which had been added a sufficient volume of D_2O-NaCl solutions to make up a final volume of 6 milliliters at a density of 1.063 gms/ml.

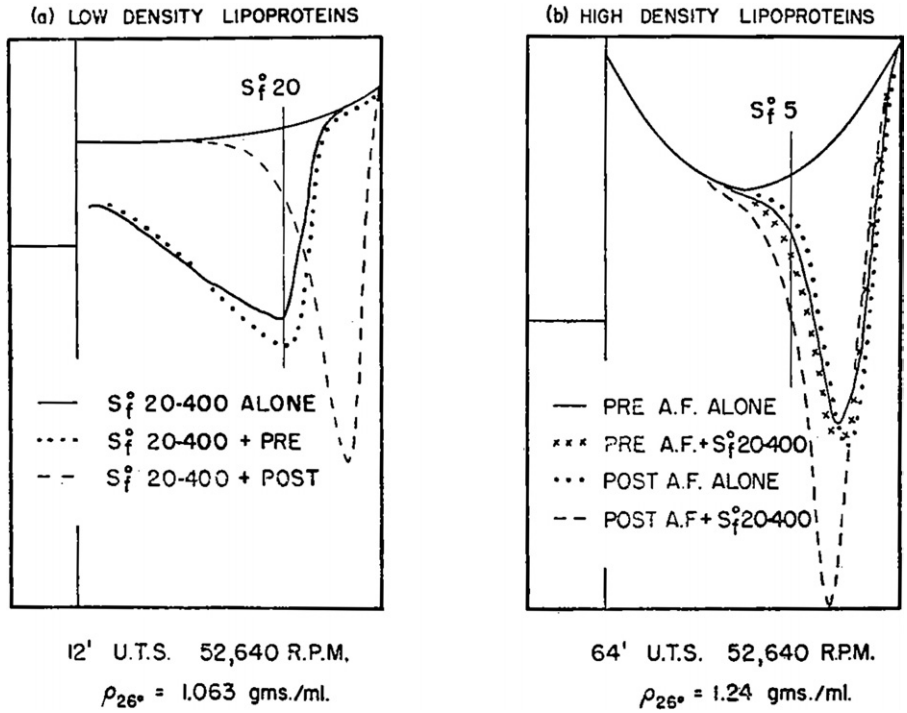

Figure 5 In vitro transformations of $S_f°20-400$ lipoproteins by an "active" fraction from post-heparinized plasma. (a) Shows the low density lipoproteins. (b) Shows the high density lipoproteins.

ipated that part of the fatty acid released during transformation of $S_f°20-400$ lipoproteins would become associated with the (HDL_2 + HDL_3) lipoproteins. The expected quantity of fatty acid released during this *in vitro* incubation could well account for the observed 10% increment in (HDL_2 + HDL_3) concentration.

The question arises as to whether the *in vitro* produced $S_f°0-20$ lipoproteins are comparable with physiologically occurring $S_f°0-20$ lipoproteins. Physico-chemical studies of the hydrated density of the most abundant species of the $S_f°20-400$ substrate and that of the *in vitro* produced $S_f°0-20$ lipoproteins were determined by measurement of flotation rates versus solution density. By measurement of flotation rates in two solutions of different, but known, densities the density corresponding to zero migration rate can be determined by extrapolation. This density is taken as the hydrated density of the lipoprotein under study. Figure 6, which shows such determinations, indicates a hydrated density of 0.978 gms/ml for the most abundant species of the $S_f°20-400$ substrate and of 1.024 gms/ml for the most abundant species of the $S_f°0-20$ lipoproteins produced *in vitro*. Comparison with data on flotation rate versus solution density obtained for isolated human lipoproteins (obtained without heparinization) in the same S_f neighborhood (see Figure 6) indicates that the *in vitro* produced $S_f°0-20$ lipoproteins are closely similar, if not identical with physiologically occurring lipoproteins of the same flotation rates.

Similar *in vitro* transformation studies were performed utilizing isolated lipoproteins of the $S_f°0-12$ class as substrate. The data are presented in Figure 7. It is seen that no significant transformation of the $S_f°0-12$ lipoprotein class is effected either by incubation with pre-heparin bottom fraction or post-heparin "active" fraction. These findings are consistent with our experience that *in vivo* heparinization of individuals whose low density lipoprotein patterns show no significant levels of lipoproteins above $S_f°12$ produces minimal, if any, lipoprotein transformation.

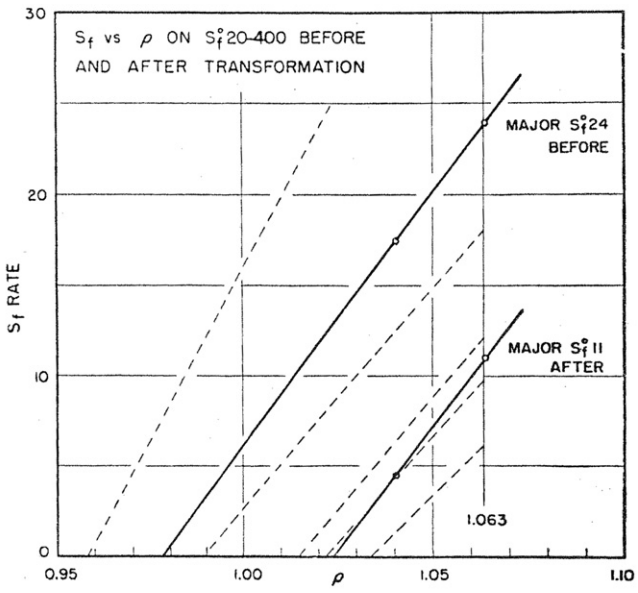

Figure 6 Flotation versus solution density study on pre-incubation $S_f°20-400$ substrate and post-incubation $S_f°0-20$ product. The extrapolated lines cross the abscissa at the respective hydrated densities of the lipoproteins (See text).

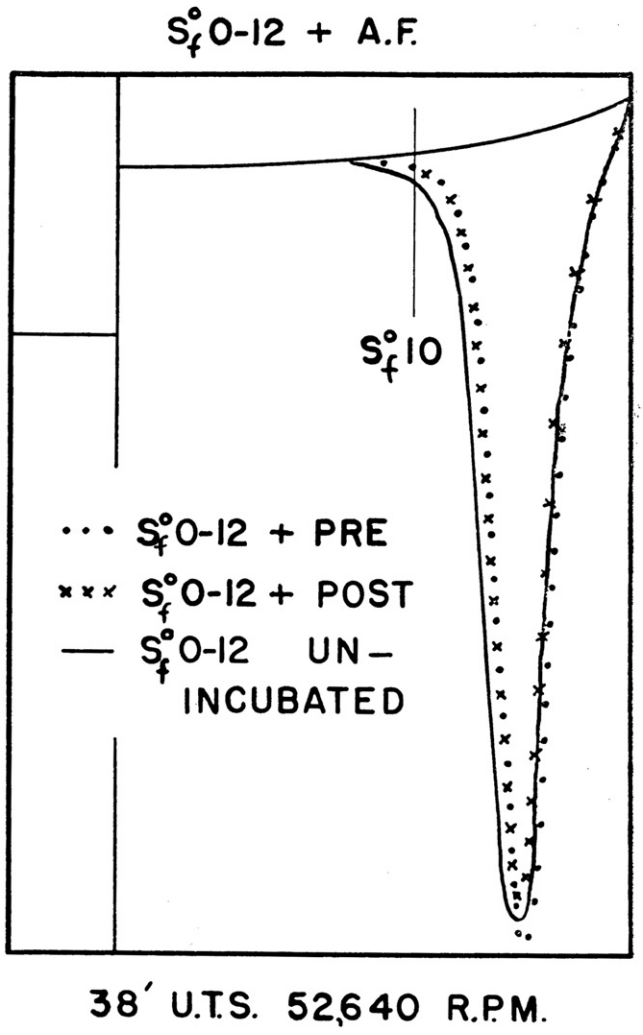

Figure 7 Comparison of $S_f°0–12$ substrate alone, after incubation with pre-heparin bottom fraction, and after incubation with post-heparin "active" fraction. Note the insignificant alteration in $S_f°0–12$ lipoproteins, indicating that the heparin "active" fraction affects this lipoprotein flotation class minimally, if at all.

From a variety of types of evidence, such as the sequence of events following a high fat meal,[5] the changes during therapy of diabetic acidosis (see Section III), and several experimental animal studies,[38–41] it appears that *physiologically* low density lipoproteins of high flotation rates are successively transformed to low-density lipoproteins of lower flotation rates. Such transformations are closely similar to those described both *in vivo* and *in vitro* following parenteral heparin administration. It is a matter of prime importance to know whether endogenous heparin or the heparin-activated lipolytic enzyme system are involved in physiologic lipoprotein transformations. If they are so involved, the question arises as to the extent to which endogenous heparin and/or the lipolytic enzyme system participate in the control of serum low density lipoproteins in the population at large and especially in several of the gross derangements of lipoprotein levels described in the metabolic states in Section V 1–12.

VI. The serum lipoproteins in clinical coronary heart disease and in coronary sclerosis

A vast amount of evidence has been accumulated through the efforts of many workers which demonstrate highly significant disturbances in serum lipid levels in clinical coronary heart disease.[2,4,7,42,43] We shall treat this problem in two parts:

(a) The relationship of serum lipoproteins with *clinical* coronary heart disease and (b) the relationship of serum lipoproteins with *pathologically* determined coronary sclerosis.

The separation of the problem into these two phases is by no means academic. What relationships exist between lipoprotein levels and the development of clinical consequences of coronary heart disease may be treated quantitatively with no assumptions whatever concerning the pathogenesis of the anatomical features of the disease. In this way *no dependence* exists upon presumed relationships between the pathologic findings and the clinical findings, leaving the clinical correlations to stand upon their own merits.

I. Clinical coronary heart disease

General considerations

In a series of previous studies we have demonstrated that both the $S_f°0–12$ and $S_f°12–400$ lipoprotein classes are significantly elevated in patients with documented myocardial infarction when compared with controls (individuals without overt coronary heart disease) matched by age and sex.[7,28,29] These data are summarized in Table 38.

It is clear from the data of Table 38 that at least for the myocardial infarcts at age 40–59 years, both $S_f°0–12$ and $S_f°12–400$ lipoproteins are significantly elevated above the levels in matched controls. The lesser difference, not statistically significant within these data, for the 60–69 year age group, is to be noted and will be commented on specifically below. For application of the findings with respect to $S_f°0–12$ and $S_f°12–400$ lipoproteins it is essential to have some method of incorporating both findings into a composite measure which takes cognizance of the differential importance of the two classes of lipoproteins with respect to coronary heart disease. The application of the statistical method known as Fisher's Linear Discriminant analysis allows assessment of the relative importance of the two lipoprotein measurements for the segregation of myocardial infarcts from matched controls. Our most recent evaluation[45] has yielded the result that the $S_f°12–400$ lipoproteins are 1.6 times as important as the $S_f°0–12$ lipoproteins, milligram for milligram, in the separation of myocardial infarcts from matched controls. A useful approach in referring to the concentration of lipoproteins with respect to

Table 38 $S_f°0–12$ and $S_f°12–400$ Lipoprotein Levels in Myocardial Infarction and in Matched Controls. (All patients with myocardial infarction were studied at least 8 weeks beyond the acute episode)

Males	Mean value $S_f°0–12$ (Myocardial Infarct) mg/100 ml	Mean value $S_f°0–12$ (Matched controls) mg/100 ml	Δ *	Significance Test	Mean value $S_f°12–400$ (Myocardial Infarcts) mg/100 ml	Mean value $S_f°12–400$ (Matched controls) mg/100 ml	Δ*	Significance Test
40–49 yrs. n = 41	427	373	+54	$P < 0.01$	345	245	+100	$P < 0.01$
50–59 yrs. N = 82	412	386	+26	$P < 0.01$	321	245	+76	$P < 0.01$
60–69 yrs. n = 37	396	378	+18	N.S.	250	230	+20	N.S.

(*) Δ = Mean Value in Myocardial Infarct Group − Mean Value in Control Series.

coronary disease is to use the term "effective concentration" in such a manner as to incorporate the 1.6 fold importance factor of the $S_f°12–400$ lipoproteins relative to the $S_f°0–12$ lipoproteins:

Thus we may write:

α = "effective concentration" of lipoproteins.

$S_f°0–12$ = concentration in mg/100 ml of $S_f°0–12$ lipoproteins

$S_f°12–400$ = concentration in mg/100 ml of $S_f°12–400$ lipoproteins

$\alpha \equiv (0.1)\,(S_f°0–12) + 0.16\,(S_f°12–400)$ (*).

α values are directly calculated for each subject by the above equation from the $S_f°0–12$ and $S_f°12–400$ lipoprotein levels. In Table 39 are given the mean α values for myocardial infarcts and for matched controls and in Table 40 are presented the distribution of α values for 50–59 year old male myocardial infarcts and for their matched controls.

It is to be noted that there exists considerable overlapping in the α value distributions for the infarct group and the matched controls. Many investigators have apparently been disturbed by the fact that such overlapping exists between these two groups. This disturbance, which has seriously interfered with progress in this field, is simply based upon a failure to understand the fundamental nature of the problem. Overlapping is, indeed, exactly what should be anticipated if those individuals who develop myocardial infarction do not show a significant change in α value simply as a result of having had an infarction. Data already published[5] indicate that such a change is not to be anticipated. The finding of a higher mean α value and a shift in the distribution of α values toward high levels in myocardial infarction patients as compared with matched controls is subject to only two possible interpretations that are free of inference or pre-conceived notions:

(a) The higher the α value in an individual, the greater is his chance of developing clinically manifest coronary disease, or

(b) The α value increases after a myocardial infarction (*). However interpretation (b) is not correct, as mentioned above, and hence interpretation (a) must be correct. Thus the difference in α values between patients with myocardial infarction and those without such overt disease provides unequivocal evidence that the higher the α value in the latter group, the higher will be the incidence rate of coronary disease.

It is, then, of importance to determine whether or not the α value alone is capable of explaining the known clinical evidence with respect to morbidity and mortality from coronary heart disease. For example age and sex variation in the incidence of coronary heart disease must be explainable from α value measurements, or some additional factor must be sought which, in combination with the α value, will allow such explanation.

In order to test the ability of the α function for predicting these differences in morbidity from coronary heart disease, it is necessary to measure coronary disease incidence rates of the various population subsegments on the basis of α values and to compare these rates with established clinical or actuarial data. The incidence (or mortality) rate per year for a particular α value is simply the ratio of the number of people with that α value who develop clinically manifest disease (or die of the disease) per year to the total number of people who have that α value. A curve of incidence rate versus α value can be constructed in this manner. This however would require for accuracy, the sampling of a large number of individuals who would have to be followed up over a period of years. This time lapse can be circumvented by construction of a curve of relative incidence rates versus α value by using a random sample of people who already have manifested clinical coronary disease by having had a

*0.1 and 0.16 are used rather 1.0 and 1.6 in order to achieve a convenient scale of values for α. This choice is purely arbitrary.

*When reference is made to "after a myocardial infarction", it is to be understood that studies are made at least 8 weeks after the episode.

Table 39 Mean α values for Myocardial Infarction Cases and For Matched Controls [$\alpha = 0.1\ (S_f°0–12) + 0.16\ (S_f°12–400)$]

Males (Myocardial Infarction) age group	Mean α value	Mean α value for matched controls	Difference in mean α values Myocardial Infarction Matched controls
30–39 yrs. (5 cases)	114.0	72.0	42.0
40–49 yrs. (41 cases)	97.9	76.5	21.4
50–59 yrs. (82 cases)	92.6	77.8	14.8
60–69 yrs. (37 cases)	79.6	74.6	5.0

myocardial infarction. The assumption inherent here, of course, is that these individuals are not significantly different from those who will come from the population in the long term study so far as α is concerned. As mentioned previously this assumption has been shown valid in a preliminary study.

The scale of relative incidence rates is constructed by dividing the number of people with a particular α value in the manifest coronary disease group by the number in the group without manifest disease who have that α value. If the actual incidence rates for a pair of α values differ by a factor of two, so will these relative incidence rates.

With such a scale of relative incidence rates it is possible to test how well the α function predicts the course of coronary heart disease in the population. This can be done by dividing the population sample into age and sex groups and comparing with established actuarial data their relative incidence rates (which are their *mean* relative incidence rates) calculated on the basis of the α value.

Incidence rates calculated on the basis of α value were found to fall short of predicting the age and sex trends in coronary disease. As a matter of fact they predict, in males, the same incidence rate at 45 years as at 65 years. It is therefore apparent that some factor in addition to the α values is needed to predict the course of the disease in the population.

As was noted earlier, the mean α value for the group with manifest coronary disease decreases with increasing age. This means that something during the intervening period between 40 and 60 years has increased the hazard associated with a particular α value since on the average the individuals in the group with manifest coronary disease have lower α values at 60 years of age than those at 40 years (see Table 39).

The simplest hypothesis which uses these facts is that it is not only the α value that determines an individual's outlook for development of overt coronary disease but also the length of time he has had that α value. Expressed more succintly an individual *accumulates* coronary disease at a rate that is proportional to his α value. If a person has an α value equal to α_1 for 10 years, he accumulates $(\alpha_1 \times 10)$ units of coronary disease; and if during the next 10 years his α value is α_2, he accumulates $(\alpha_2 \times 10)$ units in this 10 years. Over the 20 year period he would accumulate $(\alpha_1 \times 10) + (\alpha_2 \times 10)$ units of coronary disease. In the symbolism of the calculus:

$$\text{A.C.D.} \equiv \int \alpha \, dT$$

where A.C.D. ≡ accumulated coronary disease

T ≡ time, essentially a measure of age

A.C.D. therefore, is equal to the area under the curve of an individual's α values plotted against age as shown in Figure 8. In order to test the validity of this hypothesis concerning the accumulation of coronary disease one needs to calculate the A.C.D. value for each member of the population sample and to construct a curve of relative incidence rates based on A.C.D. values.

Since a plot of α versus age was not available for the members of our population sample, for purposes of calculation of A.C.D. values the reasonable approximation was made that each person, on a scale of α values, maintained the same position relative to his fellows at all ages throughout life. The A.C.D. value for each member of our population sample was calculated in this way and a scale of relative incidence rates was constructed in the manner previously described.

The distribution of A.C.D. values for individuals without overt coronary disease used to construct the scale of relative incidence rates is that for males and females between 30 and 69 years of age, combined in such proportions as to give a composite group comparable to the living population in the

Table 40 Distribution of α values for 50–59 year old Myocardial Infarction Cases (Males) and for Matched Controls

α Value	Myocardial Infarction	Matched Controls
20–29	0	3
30–39	0	19
40–49	1	45
50–59	4	82
60–69	9	106
70–79	14	111
80–89	11	105
90–99	12	76
100–109	8	52
110–119	10	17
120–129	8	22
130–139	3	11
140–149	2	8
150–159	0	2
	Total 82 cases	Total 659 cases

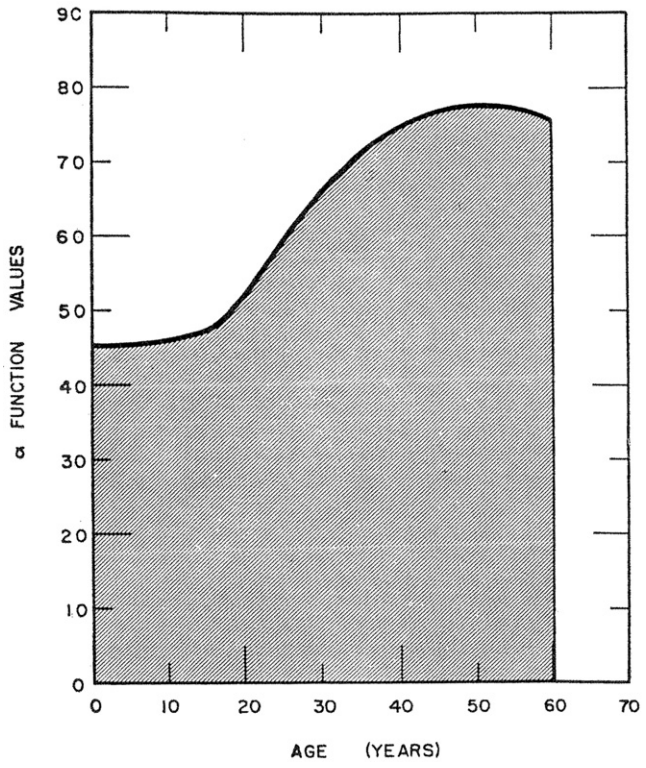

Figure 8 This is a plot of the α value for the average male through the first sixty years of life. The crosshatched area divided by 10 out to any age gives the A.C.D. value for that age.

United States in that age range (*). The A.C.D. values for individuals with coronary disease are those obtained for our entire sample with documented myocardial infarction, including males and females in the 30–69 year age range. In Table 41 are listed the distribution of A.C.D. values in our population samples of males and females considered by 10 year intervals for the range 30–69 years. In addition, in Table 41, are listed the predicted relative coronary disease incidence rates for the midpoint of each A.C.D. range considered. Figure 9 provides a curve of relative coronary disease incidence rates over the entire range of A.C.D. values. The mean incidence rate (or, simply, the incidence rate) for each of the age and sex categories of Table 41 is calculated in the usual manner for determination of mean values, i.e., the sum of the incidence rates for all individuals in the group divided by the number of individuals in the group.

No satisfactory actuarial data are available for incidence rates of coronary heart disease. However if we make the reasonable assumption that the mortality rate is the same fraction of the incidence rate in all groups, we can regard the calculated incidence rates as calculated coronary mortality rates and compare them directly with actuarial coronary mortality rates.

*The appropriate combining proportions were obtained from vital statistics data on composition of the U.S. population (1949 data).

The relative coronary disease mortality rates for the various subsegments of the population estimated as above from A.C.D. measurements are given in Table 42 together with U.S. Vital Statistics data on Mortality (**).

In order to bring both the predicted and the Vital Statistics mortality rates on to the same scale of relative values, the mortality rates for one group (arbitrarily chosen as the 44.3 year old males) were set at unity both for predicted and actuarial values. The general agreement between the mortality rates predicted solely upon A.C.D. values and those obtained from Vital Statistics data is striking. The overall quantitative aspects of the age and sex trends in coronary disease mortality are predicted well from A.C.D. values. First, the higher mortality in the male sex as compared with the female sex is predicted. Second the rate of increase in mortality with increasing age is essentially in complete quantitative agreement for males, and except for the slightly high prediction in the youngest groups, is also in quantitative agreement for the female.

Overweight and coronary heart disease

Overweight has been implicated as a major factor associated with an excessive incidence of mortality from coronary heart disease. Dublin[46] and Dublin and Marks[47] have quantitated the excessive coronary disease mortality rates in overweight individuals. For coronary heart disease, Dublin's data show a 1.6 fold increase in coronary mortality, for example, for individuals 40% above ideal weight. It is of interest to know whether this general increase is predictable from the A.C.D. concept of the evolution of coronary disease. For population samples, α values and A.C.D. values can be calculated from lipoprotein measurements for each individual, for whom relative weight is also available. Since each A.C.D. value corresponds to a particular value of relative coronary disease mortality rates, it is possible to construct a relationship between relative weight values, for a particular age and sex category, and estimated relative coronary disease mortality rates. Such a relationship is presented in Figure 10. Comparison of the relative coronary disease mortality rates for individuals at 40% overweight contrasted with individuals at ideal weight gives a ratio of coronary mortalities of 9.2/5.7, or 1.6. This is in good general agreement with Dublin's measured value for this comparison, 1.6. It therefore seems likely that the A.C.D. hypothesis accounts at least for the major aspect of the relationship of overweight with coronary heart disease.

Diabetes Mellitus and Coronary Heart Disease

Diabetes mellitus is widely quoted as a metabolic disorder associated with excessive coronary heart disease. Yet no

**The following categories of death in the Vital Statistics tables have been considered to be representative of coronary heart disease; 420 (arteriosclerotic heart disease, including coronary heart disease), 422 (other myocardial degeneration), 440–443 (hypertension with heart disease) (1949 U.S. Vital Statistics).

Table 41 Distribution of A.C.D. Values in Population Samples and the corresponding Predicted Relative Coronary Disease Incidence Rates. Number of Individuals in Each A.C.D. Range, by Age-Band and Sex

Range of A.C.D. Values	Males 30–39 years	Males 40–49 years	Males 50–59 years	Males 60–69 years	Females 30–39 years	Females 40–49 years	Females 50–59 years	Females 60–69 years	Corresponding Predicted Relative Coronary Incidence Rate
0–50	0	0	0	0	0	0	0	0	
50–100	17	0	0	0	33	1	0	0	< 0.00001
100–150	95	12	2	0	155	47	4	0	0.00001
150–200	112	57	10	2	106	105	41	5	0.0003
200–250	72	91	34	6	49	88	56	18	0.011
250–300	41	77	53	19	23	46	65	59	0.062
300–350	10	47	58	26	7	18	55	54	0.115
350–400	7	19	47	38		8	21	13	0.185
400–450	4	10	30	34		3	11	15	0.300
450–500		0	10	22		0	7	5	0.480
500–550		1	8	9		1	2	3	0.720
550–600		1	6	9				5	1.150
600–650			1	9				3	1.900
650–700				2					3.00
700–750				1					4.60
750–800				0					7.50
800–850				1					12.00
TOTAL	358	315	259	178	372	317	262	180	
Mean Age in Years	35.1	44.3	54.2	62.1	35.2	44.0	53.9	62.1	

satisfactory data are available for cross-sections of the diabetic population with which incidence rates of coronary heart disease can be calculated. Further any such data collected in the past twenty-five years are subject to the objection that the diabetics in such a study may represent a mixture of pre and post insulin era diabetics. Furthermore a test of the extent to which the A.C.D. concept accounts for any alteration in incidence of coronary disease in diabetes mellitus would require knowledge of whether significant lipoprotein alterations occur at the time of actual onset of the diabetes, or whether any lipoprotein abnormalities had characterized the diabetics before the onset of clinically manifest diabetes. Some theoretical predictions, however, can be suggested from the study of diabetics who pass through an episode of clinical acidosis. In the series of 10 cases of diabetics in acidosis, there was a marked derangement in serum lipoprotein levels with a great resultant increase in α value. The mean alpha value for this series of 10 cases in acidosis was 240 units; which is extremely elevated. Seven of the 10 cases showed α values above the extremely high value of 140 units. Further the study of some of these cases during the period of re-control of the diabetes following the acidotic episodes shows a marked amelioration of the lipoprotein derangement with correction of the acidosis. While the precise factors, during acidosis, which lead to lipoprotein derangement require much further elucidation, it would be estimated that diabetics with the serum lipoprotein derangement of acidosis would be accumulating coronary disease at a greatly enhanced rate. Hence, in an appreciable period of time spent with such a derangement, total accumulated coronary disease would be greater and would be accompanied by a greater clinical coronary heart disease incidence rate.

Other clinical states and coronary heart disease

In the preceding sections several other metabolic states have been shown to be associated with one or another lipoprotein class highly elevated. Among these are myxedema, xanthelasma, xanthoma tendinosum, xanthoma tuberosum, nephrotic state and essential hyperlipemia. The mean α values for these various groups are presented in Table 43.

From the α values estimated in Table 43 it is evident that the *rate* of accumulation of coronary disease is excessive for the various disorders described. An estimate of the predicted coronary disease incidence rate based upon the concept of accumulated coronary disease would be dependent upon knowledge of the fraction of the life span during which the disorder had been manifest. Such information, in precise form, is not available, and would probably best be evaluated on individual cases. Certainly during the period of years that an individual demonstrates the lipoprotein derangement of such disorders, coronary disease may be anticipated to have

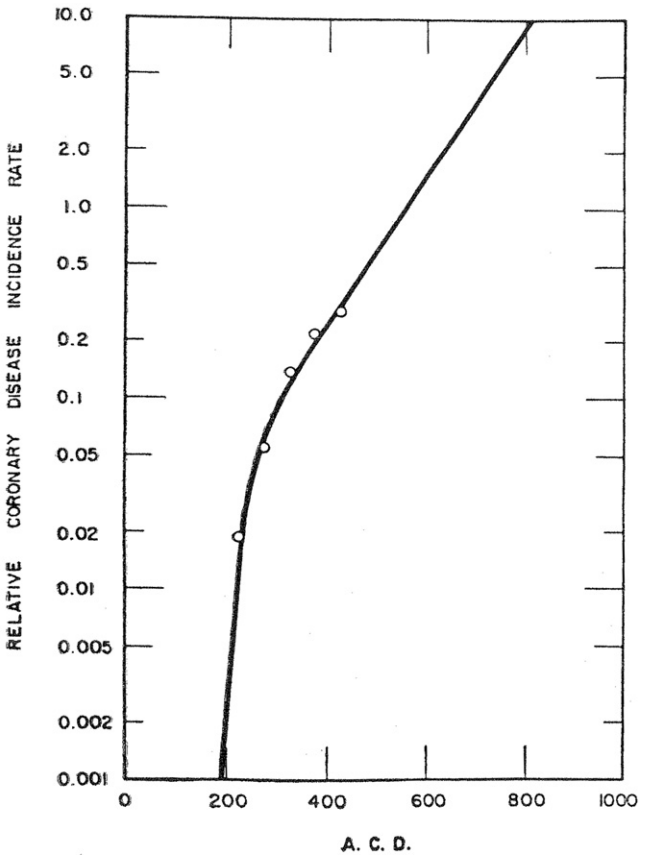

Figure 9 Relative Coronary Disease Incidence Rate plotted as a function of A.C.D. values (accumulated coronary disease values). This curve is based upon the distribution of A.C.D. values in the non-coronary and coronary populations studied. (See text for derivation).

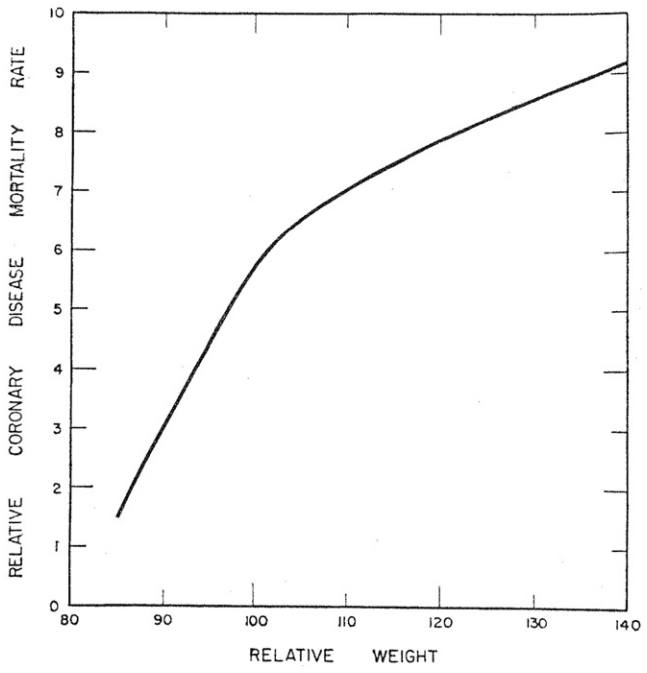

Figure 10 The Relationship between Predicted Coronary Disease Mortality and Relative Weight. (Based upon A.C.D. calculations for a population sample of 40–49 year old United States males).

accumulated at a excessive rate. Semi-quantitative clinical estimates suggest earlier development of clinical coronary heart disease in most of these states, which is in accord with the predictions from A.C.D. evaluation.

Within the framework of the experimental and clinical data upon which the predictions are necessarily based, the observed agreements with actuarial data may be considered to support the relationship of the concept of accumulated coronary disease, as derived from lipoprotein measurements, with the clinical occurrence of coronary disease. A first-order concept of the nature of the quantitative evolution of coronary disease is thus available. Second-order refinements are to be anticipated. Probably the most important such refinement will be a determination of the precise magnitude of the independent contribution of the blood pressure level in coronary disease. Elsewhere evidence was presented[44] that the diastolic blood pressure is involved in coronary disease, and is so involved independent of its interrelationship with serum lipoproteins. The best approach to the quantitative integration of the blood pressure findings into the concept of "accumulated coronary disease" requires further evaluation.

Table 42 Comparison of Predicted Coronary Disease Mortality Rates with Those from U.S. Vital Statistics (*)

	Mortality Rates from U. S. Vital Statistics	Predicted Mortality Rates from A.C.D. values
Males		
35.1 years	0.27	0.32
44.3 years	1.00	1.00
54.2 years	3.39	2.94
62.1 years	6.73	7.99
Females		
35.2 years	0.06	0.12
44.0 years	0.26	0.46
53.9 years	1.06	1.41
62.1 years	2.97	2.94

(*) United States Vital Statistics Tables, 1949 data.

Table 43 Mean α Values for Several Lipoprotein Transport Disturbances

Disease Category	Mean α Value in Disease	Mean α Value for Matched Controls
Nephrotic State	266.5	51.1
Essential Hyperlipemia	260.2	78.0
Xanthoma Tuberosum	243.6	76.0
Xanthoma Tendinosum	129.5	67.7
Spontaneous Myxedema	123.5	74.9
Xanthelasma	87.8	71.4

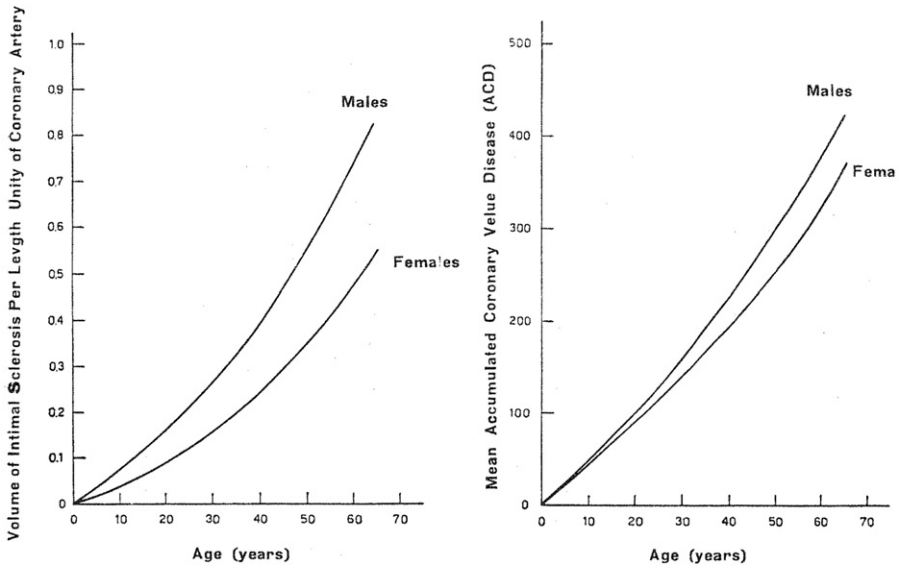

Figure 11 (a) Plotted here are the mean values of volume of sclerotic intimal tissue per unit length of coronary artery as related to age (Calculations made from Lober's pathologic-anatomic data). (b) Plotted here are the mean Accumulated Coronary Disease (A.C.D.) values for a representative United States population sample. (Calculated solely from ultracentrifugal lipoprotein measurements). The general agreement in the character of both plots is noteworthy.

(b) Pathological aspects of coronary heart disease

The existence of a pathologic-anatomic feature in coronary heart disease has been recognized for many years.[48,49] This feature is coronary artery atherosclerosis. It is important to know if and how the concept of "accumulated coronary disease" derived from clinical correlations alone is related to the pathologic features of coronary sclerosis.

From the clinical and biochemical studies the A.C.D. function is characterized by the following outstanding properties:

(a) Within each sex, there is an increase in mean A.C.D. value with increasing age, for individuals without overt coronary heart disease.
(b) At least out to the age of 70 years, the mean A.C.D. value is lower for the female sex than for the male sex in the groups without overt coronary heart disease.
(c) The distribution of A.C.D. values shows appreciable overlapping between a group of individuals with overt coronary heart disease and an otherwise comparable group without overt coronary heart disease.

If a pathologic-anatomic feature is to be considered as a primary factor underlying the clinical evolution of coronary heart disease, this feature must have properties consistent with those for the A.C.D. value which has been shown above to be consistent with the clinical features of coronary heart disease. To determine whether coronary artery atherosclerosis fulfills the requisite criteria, a quantitative measure of *degree* of coronary artery involvement with atherosclerosis is necessary. The pathogenetic sequence of development of such features as lipid deposition, fibrosis, calcification, thrombosis, and narrowing in coronary atherosclerosis is not known with certainty at this time. It appears reasonable to us that the present best estimate of the degree to which pathological coronary disease has progressed in an individual is represented by the area of the vessel involved with atheromatous tissue, irrespective of whether that be lipid, fibrotic, calcific, etc. What is needed is a measure of such degree of involvement in populations comparable to those for which A.C.D. evaluations have been made. No ideal data, free from possible biasing features and entirely comparable with our population with A.C.D. measurements, exist to our knowledge. The best approximation to this appears to reside in the data of Lober[50] based upon necropsy examination of hearts from hospital deaths. From Lober's data we have calculated the area of intima involved with tissue, which should correspond closely with the above described area of vessel involved with atheromatous tissue. These calculated values are plotted in Figure 11. In Figure 11 are presented our own mean population values for the A.C.D. function. The calculated values for degree of coronary involvement with atheromatous tissue show the following consistencies with the A.C.D. function:

(a) Both show, for each sex, an increase in mean value with increasing age for individuals without overt clinical coronary disease. Further the slopes of increase of both values with aging are comparable.
(b) Both show, for the age range through 70 years, a lower value in the female sex than in the male sex.
(c) Whereas Lober did not present distribution of sclerosis at a given age, there are data from Spain,[51] White, Edwards, and Dry,[52] and Ackerman, Edwards and Dry,[53] which show a distribution of degree of involvement at a given age from low through high values, with some individuals in the population at large showing degrees of involvement higher than the average for

groups with clinically manifest coronary disease. This is consistent with the overlap in distribution of A.C.D. values between populations with and without clinically overt coronary heart disease.

The general trends in values for degree of atheromatous involvement of the coronary arteries are consistent with those for the A.C.D. function. This suggests that degree of involvement, estimated in the manner above, is a pathologic feature closely related to the clinical aspects of coronary heart disease. Further the suggestion is implied that lipoprotein measurement at a particular age allows estimation of the average involvement of the coronary arteries with atherosclerotic tissue.

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