THE CANADIAN MEDICAL ASSOCIATION

LE JOURNAL DE

L'ASSOCIATION MÉDICALE CANADIENNE

NOVEMBER 9, 1963 • VOL. 89, NO. 19

Studies of Male Survivors of Myocardial Infarction due to "Essential" Atherosclerosis

II. Lipids and Lipoproteins

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ABSTRACT

Serum lipids (total, ester and free cholesterol, phospholipid and standard S_f 0-12, 12-20, 20-100 and 100-400 lipoproteins) were determined in 102 men, ages 30-70, with atherosclerotic coronary heart disease (C.H.D.), free of hypertension, diabetes or other complicating variables. All the lipid fractions had a lognormal distribution. All were significantly higher than in the controls up to the seventh decade. An explanation for the declining serum lipid levels with age was found. Contrary to previous reports, there was no abnormality in the relation of various lipid fractions to one another in C.H.D. A spurious correlation between C/P ratio and total cholesterol was found in both groups. In separating coronary and control subjects, cholesterol and 0-12 lipoproteins were the most reliable criteria and were equal in this respect. The misclassification rises from 20% in the fourth to 33% in the seventh decade. C/P ratio offered no improvement. The triglyceride-containing 100-400 lipoprotein was an inferior discriminator. Employing all the lipid fractions, discriminant analysis provided a minimum 12% misclassification in the fourth decade. There was no demonstrable relationship of cholesterol to body measurements, physical activity or family history of C.H.D.

IN CANADA, as in other highly developed countries, atherosclerotic coronary heart disease (C.H.D.) is the leading cause of death. Because of this, a long-term comprehensive investigation of persons with this disease was organized. A previous communication[1] described in detail the purposes and procedures of the study and dealt with the characteristics of the patients. In brief, all were men who had survived a myocardial infarction due to "essential" atherosclerosis, i.e. atherosclerosis unaccompanied by any condition believed to be an aggravating factor, such as diabetes or hypertension. The purpose of this selection was to examine the relationship of the serum lipids alone to C.H.D.

This second report compares the serum lipid and lipoproteins of the C.H.D. patients with those of healthy subjects and examines the relative abilities of these fractions to discriminate between the two groups. The relation of the various lipid fractions to one another, and to age, body measurements, physical activity and family history of C.H.D., was also evaluated. Some of the data to be reported here have appeared in interim abstracts.[2-4] The results in the control subjects have already been published.[5]

Clinical Material

Selection

The subjects, all men, were chosen from the files at Sunnybrook (Department of Veterans Affairs) Hospital in Toronto.

The coronary patients had unequivocal evidence, anamnestic and electrocardiographic, of ischemic heart disease as manifested by one or more myocardial infarctions. Any with valvular disease, syphilis, anemia or polycythemia, thromboangiitis obliterans or polyarteritis nodosa were excluded, so that the cause for the ischemia was in every instance almost certainly atherosclerosis. The controls were taken at random from files classified under minor diseases thought not to affect the variates to be studied.

From the Atherosclerosis Project, Sunnybrook (D.V.A.) Hospital, Toronto, and the Department of Medicine, University of Toronto. Aided by a grant from the Ontario Heart Foundation.

Both groups were selected in such a way as to be free of hypertension, arbitrarily defined as the persistent elevation of the blood pressure above 150/90 mm. Hg. The availability of military and medical documents afforded the unique opportunity to recognize and exclude those coronary patients with normal blood pressure levels who had hypertension preceding their infarction. There were no important differences in blood pressures between the two groups or between various decades within each group.

All subjects in the study were also free of diabetes, nephrosis and hypothyroidism, diseases commonly associated with secondary hypercholesterolemia. The presence of xanthomatosis in a patient or in his relatives was not cause for exclusion. Nor was any attempt made to exclude essential hypercholesterolemia or triglyceridemia for which satisfactory diagnostic criteria are not as yet established. The presence of any major disease, especially those with important metabolic consequences (hepatobiliary disorders, endocrinopathies, gout or cancer and other wasting diseases), was reason for exclusion from both groups. Any person in either group receiving a special diet or treatment with hormones or anticoagulants was eliminated from the study. All the subjects were ambulant, having been discharged from hospital for at least three months. This criterion was adopted to eliminate the changes that occur in the serum lipids and lipoproteins in the first weeks following myocardial infarction.[6, 7]

To allow for a satisfactory statistical evaluation, some 25 men in each decade, for each of the two groups, were assembled. The coronary group was selected first and a control group was then matched by age. The rate of refusal to participate was less than 10% and was similar in the two groups, so that no significant bias was introduced from this source. It is of interest that only a small fraction of the clinical coronary population could meet the rigorous requirements outlined above.

Procedure

After an overnight fast, each subject, coronary and control, was investigated by the authors. The investigation included basal metabolism tests; determination of lipids and lipoproteins on fasting blood specimens drawn between 8.00 and 8.30 a.m. to eliminate the effect of diurnal variation; detailed family, medical and dietary history; complete physical examination; fluoroscopic and orthodiagraphic examinations; 12-lead electrocardiogram; posteroanterior and left lateral chest radiographs; and lateral radiographs of the abdomen for the detection of aortic calcification. A urinalysis, hemogram, determination of blood sugar and non-protein nitrogen levels and a Wassermann reaction were also performed on each subject. Where indicated, determinations of serum protein-bound iodine, radioactive iodine uptake, glucose tolerance test, liver function tests and fasting electrocardiographs were done.

LABORATORY METHODS

Serum lipid determinations for each man were performed on a single fasting blood specimen. Total and free serum cholesterol were estimated in duplicate by a modified Schoenheimer and Sperry method;[8] the phospholipid in duplicate by the method of Zilversmit and Davis.[9] All of the cholesterol and phospholipid determinations were done by one biochemist. The duplicates were done simultaneously but were not blind. Serum lipoproteins were determined by the technique of de Lalla and Gofman[10] in the Ultracentrifuge Laboratory, McGill University, Montreal.

The standard technical errors for the determinations of each serum lipid fraction have been given previously[5] and compare favourably with those from other laboratories.

COMPOSITION AND CHARACTERISTICS OF THE GROUPS

The number and ages of the men in the two groups are shown in Table I. There are approximately 25 patients in each decade and the mean ages are similar. It is emphasized that this coronary group, by arbitrary selection (Canadian, male, military veterans, survivors of previous myocardial infarction, with no aggravating disease and no associated disease), is not representative of the coronary population as a whole. Moreover, the choosing of an equal number of patients in each decade (to facilitate stratified comparisons) greatly alters the age distribution from that seen in an unweighted series. For example, in Cassidy's series of 1000 coronary patients, those in the fourth and fifth decade comprised only 3.2 and 14.6%, respectively, of the total number.[11] It is clear then that the results of the appraisal of this coronary group need not necessarily apply to the general coronary population.

TABLE I.—COMPOSITION OF THE GROUPS: NUMBER AND AGE

	Control			*Coronary*		
Decade	*n*	*Mean age*	*s*	*n*	*Mean age*	*s*
Fourth	25	34.4	2.9	25	36.3	2.8
Fifth	25	43.6	2.7	26	44.5	3.0
Sixth	27	56.0	2.4	28	55.5	2.4
Seventh	23	64.1	2.8	23	64.3	2.7
	100			102		

The characteristics of the patients and their controls have been described in detail.[1] In both groups, more than half were born in Canada; most of the remainder had come from the British Isles in their youth. There was one Jew but no Negroes. Various occupational classes were satisfactorily represented and similar. A large proportion of the coronary population was judged in the "minimal physical activity" category at the time of investigation.

A dietary survey[12] showed that the mean daily calorie intake in the coronary group decreased progressively from 2250 in the fourth to 1612 in the seventh decade. In the control group, there was the same trend at a higher level of intake: 2565 calories in the fourth to 2148 calories in the seventh decade. However, in each decade of both groups, the average fat consumption was approximately 38% of the total calories. The coronary group tended to eat a smaller amount of milk-fats and eggs.

RESULTS AND DISCUSSION

Individual Values

The results of the serum lipid fractionations on each individual in the coronary group are recorded in the Appendix to this report.

Frequency Distributions

In the control group,[5] total, ester, and free cholesterol, phospholipid and standard (Std.) S_f 0-12 lipoprotein had normal distributions. Std. S_f 12-20, 20-100 and 100-400 lipoproteins were positively skewed, and their transformation into logarithms resulted in normal distributions.

The cumulative distributions of all the lipid fractions in the coronary group are plotted on probit paper in Figs. 1 and 2. A normal distribution would produce a straight line. Varying degrees of positive skewing, from slight for cholesterol to marked for Std. S_f 100-400 lipoprotein, are present as indicated by the upward concavity of these curves. As shown by the t values for the index of skewness in Table II, log transformations of each fraction result in normal distributions. In the case of lipoproteins 12-20, 20-100 and 100-400, the log transformation produced skewing that was negative but of lesser degree. Tests of kurtosis, as shown by the t values for the index of skewness in Table II, indicate that log transformations generally improve the normality of the distribution of the lipid and lipoprotein fractions in this regard as well. Therefore, it can be said that all the lipid fractions in this coronary group have a lognormal distribution. However, since this is an age-selected group (*vide supra*), the distributions of the serum lipids need not necessarily apply to a coronary population unselected for age.

In the further statistical analysis reported herein, log transformations were employed but are not reported, since they did not alter the results of the tests using the unmodified data.

TABLE II.—t VALUES FOR THE INDEX OF SKEWNESS‡ (g_1) AND INDEX OF KURTOSIS‡ (g_2) FOR EACH LIPID FRACTION IN 102 CORONARY PATIENTS

	Skewness		*Kurtosis*	
Serum fraction	$t\ g_1$	$t\ g_1$ *(log)*§	$t\ g_2$	$t\ g_2$ *(log)*§
Total cholesterol	2.57*	0.36	1.66	0.0
Ester cholesterol	2.22*	0.24	0.82	1.88
Free cholesterol	3.39**	0.71	2.70**	0.72
Phospholipid	1.96*	0.40	0.49	0.0
Std. S_f 0-12	5.02**	0.53	5.23**	1.84
12-20	3.35**	-3.14**	3.67**	1.85
20-100	3.97**	-2.18*	1.35	1.00
100-400	10.33**	-3.38**	16.14**	2.00*
12-400	4.35**	1.76	1.94	0.78
0-400	2.80**	0.13	0.65	1.03

*Significantly different from a normal distribution, p <0.05.
**Significantly different from a normal distribution, p <0.01.
‡Snedecor, G. W.: Statistical methods, The Iowa State College Press, Ames, Iowa.
§t values for the indices of the log transformed distributions.

TABLE III.
SERUM LIPIDS, ARITHMETRIC MEAN AND STANDARD DEVIATION§

		Decade			
Fraction	*Group*	*4*	*5*	*6*	*7*
Total cholesterol mg.%	Control	204.9	214.5	206.5	215.8
		±28.7	±38.0	±30.5	±36.6
	Coronary	278.9***	262.2***	250.0***	231.3
		±52.9	±32.4	±46.7	±36.0
Ester cholesterol mg.%	Control	147.5	154.8	147.8	154.1
		±20.0	±27.3	±22.9	±25.2
	Coronary	198.4***	186.8***	177.8***	165.6
		±36.8	±22.8	±34.6	±25.9
Free cholesterol mg.%	Control	57.4	60.1	58.7	61.7
		±9.1	±11.4	±8.2	±12.0
	Coronary	80.6***	75.3***	72.3***	65.7
		±17.7	±10.9	±13.1	±11.8
% free cholesterol	Control	28.0	27.8	28.4	28.5
		±1.1	±1.4	±1.4	±1.4
	Coronary	28.8	28.7	28.9	28.4
		±2.3	±1.6	±1.8	±2.0
Phospholipid mg.%	Control	242.4	255.0	244.5	260.0
		±29.0	±32.5	±30.3	±36.5
	Coronary	284.2***	280.5**	274.4***	255.4
		±47.6	±25.6	±36.1	±32.7

Significance of differences from the control group: *p <0.05 **p <0.01 ***p <0.001
§Geometric means and 95% limits of the coronary groups are in the Appendix.

TABLE IV.—SERUM LIPOPROTEINS, ARITHMETIC MEAN AND STANDARD DEVIATION, MG.%§

		Decade			
Standard Sf	*Group*	*4*	*5*	*6*	*7*
0 - 12	Control	286.0	299.6	289.1	281.1
		±40.1	±51.1	±45.5	±52.7
	Coronary	426.2***	407.9***	343.0***	327.0**
		±127.8	±103.8	±61.2	±56.9
12 - 20	Control	51.4	54.8	57.3	49.2
		±21.3	±23.1	±28.5	±21.3
	Coronary	75.4**	66.5*	60.4	47.7
		±34.6	±26.3	±21.4	±19.8
20 - 100	Control	85.2	84.2	77.8	84.0
		±41.0	±40.8	±34.4	±76.0
	Coronary	122.4*	124.0**	110.2**	69.9
		±60.4	±62.4	±51.9	±44.0
100 - 400	Control	34.3	31.1	20.8	30.3
		±31.7	±27.8	±11.6	±39.6
	Coronary	61.6*	60.3	55.6***	28.7
		±59.3	±75.9	±37.8	±31.6
12 - 400	Control	170.8	170.1	155.9	163.5
		±35.6	±75.0	±56.1	±126.5
	Coronary	259.3**	250.8**	226.1**	146.3
		±129.3	±131.2	±100.0	±86.1
0 - 400	Control	456.9	469.7	445.0	444.6
		±93.4	±104.8	±83.9	±153.1
	Coronary	685.6***	658.7***	569.1***	473.3
		±191.3	±144.2	±138.4	±115.0

Significance of difference from the control group: *p <0.05 **p <0.01 ***p <0.001
§Geometric means and 95% limits of the coronary groups are in the Appendix.

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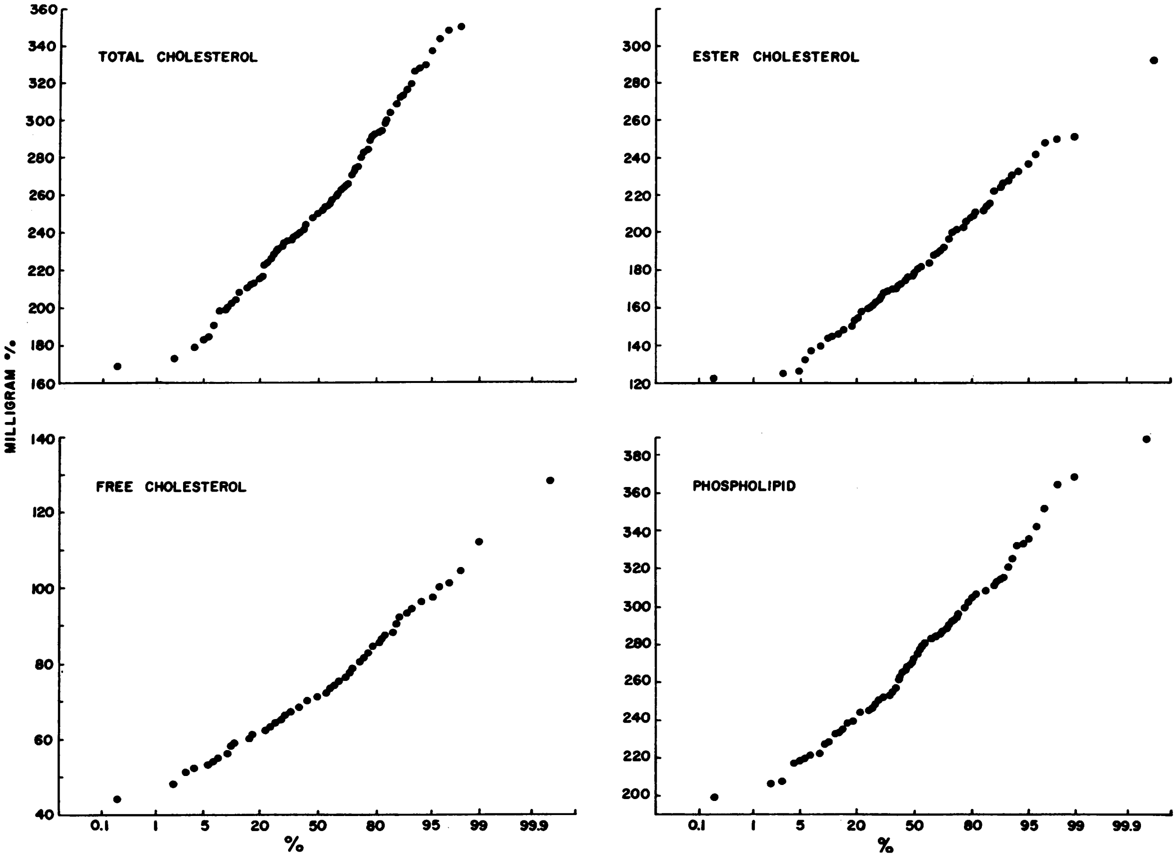

Fig. 1.—Cumulative frequency distributions (on normal probit paper) for serum lipids in 102 coronary patients.

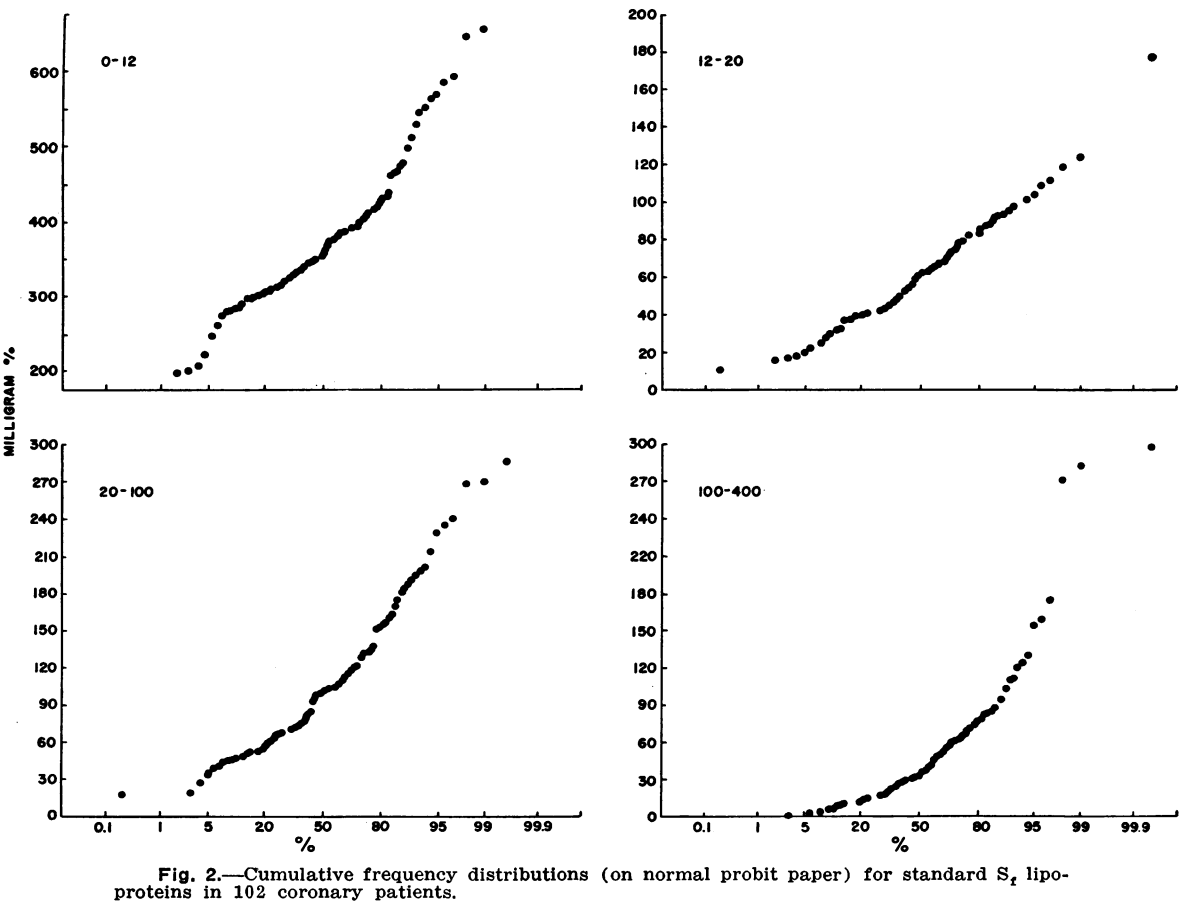

Fig. 2.—Cumulative frequency distributions (on normal probit paper) for standard S_f lipoproteins in 102 coronary patients.

Mean Values and Age

The mean values for the lipid fractions of the control and coronary groups by decade are shown in Tables III and IV and in Fig. 3. In the control group, there is no important variation with age in any of the fractions. In the coronary group, the maximal elevation of all the fractions occurs in the fourth decade. For most fractions this elevation is highly significant. The levels of all the fractions then progressively decrease with age to the seventh decade, where they are no longer significantly different from those of the control group. This decrease in the serum lipids with age was tested in the case of total cholesterol and found to be significant, the regression coefficient being -15.4 ± 3.9 (s.e.) mg. % per decade.

Atherosclerosis is present in all adults in this Western civilization. Studies such as this have been criticized as comparing coronary patients with controls who have merely a lesser degree of atherosclerosis. Surely this fact must add to, rather than detract from, the significance of a demonstration of differences in circulating lipids between the two groups. Probably the physiologic mean serum lipid levels for humans are those of primitive societies with a negligible incidence of clinical atherosclerosis, for example, total cholesterol 150 mg. %. Indeed, as previously discussed,[5] the so-called "normal" average cholesterol level of 210 mg. % in Western society probably represents a moderate form of hypercholesterolemia in regard to the association with a high incidence of clinical atherosclerosis. The lipid levels of the coronary subjects in our society could, therefore, be considered as being still more abnormally elevated.

The present results confirm the many previous reports that in countries where C.H.D. is common, groups of men with C.H.D. have higher serum lipid concentrations than their clinically healthy counterparts[7, 13-16] and that the most significant elevation of serum lipids occurs in the earlier decades. Indeed, it is only under the age of 50[6, 17, 18] that the differences are impressive.

Although some have found important differences in the serum lipids to extend into the seventh decade,[14, 16] as in this investigation, most studies found the differences to be significant up to and including the sixth decade only. This has its parallel in pathological studies in which the incidence of severe atherosclerosis increased from the fourth to a maximum in the sixth decade and decreased somewhat thereafter.[19] Paterson, Dyer and Armstrong[20] found no relationship between the level of serum lipids determined in life and the amount of coronary atherosclerosis demonstrated post mortem in men over 60 years of age.

Why the serum lipids are higher in the younger than in the older C.H.D. patients (as illustrated in Fig. 3) has been the subject of much speculation. Oliver[21] suggested that there may be at least two types of coronary disease—one associated with an

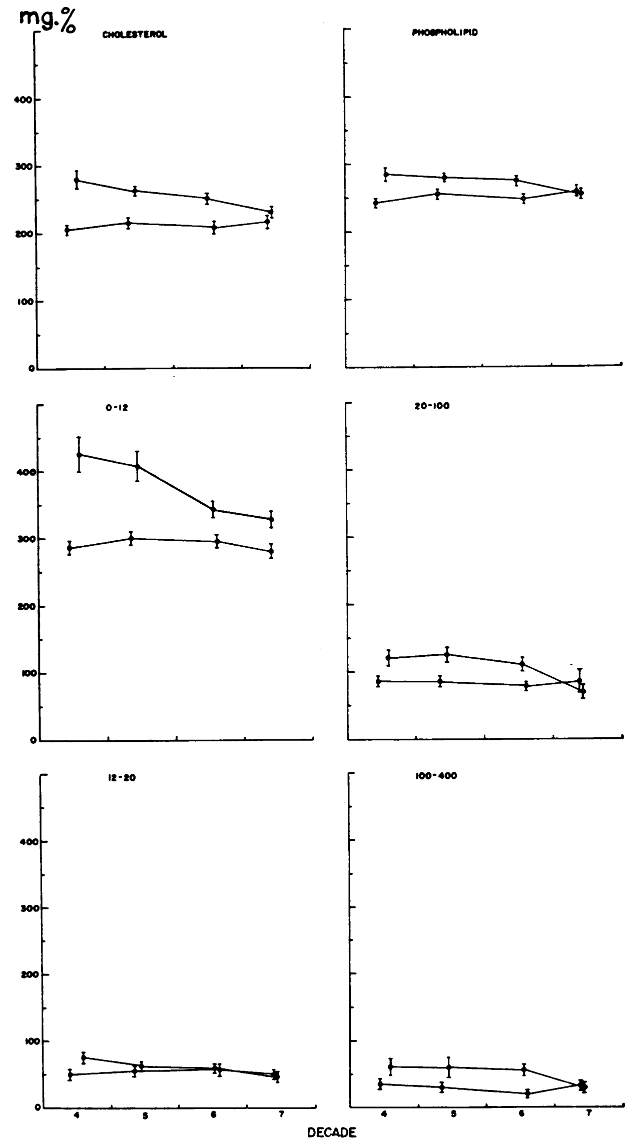

Fig. 3.—Mean serum lipid and lipoprotein values ± S.E. in each decade of the coronary (•) and control (°) group.

active metabolic disorder and another associated with advancing age. However, in cases of essential hypercholesterolemia and xanthomatosis there is a continuous transition from patients with very high cholesterol concentrations and extensive deposits, to those with moderate hypercholesterolemia and small deposits, to patients with questionable xanthomas. This latter group merged into the "ordinary" group of patients with cardiac infarction.[22] The decreasing concentrations with age of the lipids in our patients occur in a way which is progressive and thus might support the concept of a single continuous spectrum of C.H.D.

In the present study several observations help to explain the decrease in serum lipid levels in C.H.D. with age. From Fig. 3 it would appear that the higher the serum lipids, the earlier the age of onset of clinical C.H.D. Subsequent to the onset of clinical C.H.D., the high five-year mortality of 40% in each decade of this group[23] means that most coronary patients, including the young ones with

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their high cholesterol levels, die before entering the next decade. In addition, the repeated determination of serum cholesterol over a five- to seven-year period in individual survivors of a myocardial infarction has shown an average decline in serum cholesterol of 1.3 mg. % per year.[24] Thus at least three factors: (1) earlier onset of clinical disease in hypercholesterolemic subjects, (2) high subsequent mortality, and (3) decreasing cholesterol concentration with age in survivors, are involved in the effect of age on the level of serum lipids in C.H.D. It may be added that, contrary to general expectation, the survival rate in untreated C.H.D. is not related to the level of the serum lipids.[23]

Relation Between the Various Lipid and Lipoprotein Fractions

Free and Total Cholesterol

As in the control group,[5] the range of percentage of total cholesterol which is free is small, 21.3 to 33.2% (see Appendix). The mean is 28.7%. The correlation coefficient between free and total cholesterol is high ($r = .93$, $p < 0.001$). Table III shows that the mean and standard deviations are similar in all decades. It also shows that despite the higher total cholesterol levels in the coronary group, the percentage free cholesterol remains the same as in the controls.

Thus in C.H.D., as in health,[5, 25] the relation between the free and total serum cholesterol is a variable with a very small dispersion about the mean of 28%. Because of this the calculation of further ratios, such as that between the ester and free cholesterol, can only be tautologous. Nor is there anything to be gained by relating the ester or free cholesterol to any variate when the latter's relation to total cholesterol has already been established.

With either severe liver failure or triglyceridemia, free cholesterol may even exceed 50% of the total. Otherwise, a gross variation of the percentage free cholesterol outside the limits established by this and other studies[25] must be attributed to technical error. We have found the percentage free cholesterol to be one means of checking on laboratory technique. It is disconcerting when studies of C.H.D. report grossly abnormal percentage free fractions.[17]

*Serum Cholesterol and Phospholipid (C/P Ratio)**

It has been repeatedly implied[17, 26, 27] that the relationship of cholesterol to phospholipid is disturbed in patients with clinical atherosclerosis. Some have also claimed that the C/P ratio discriminates between atherosclerotic and healthy subjects better than total cholesterol.[17] Because of

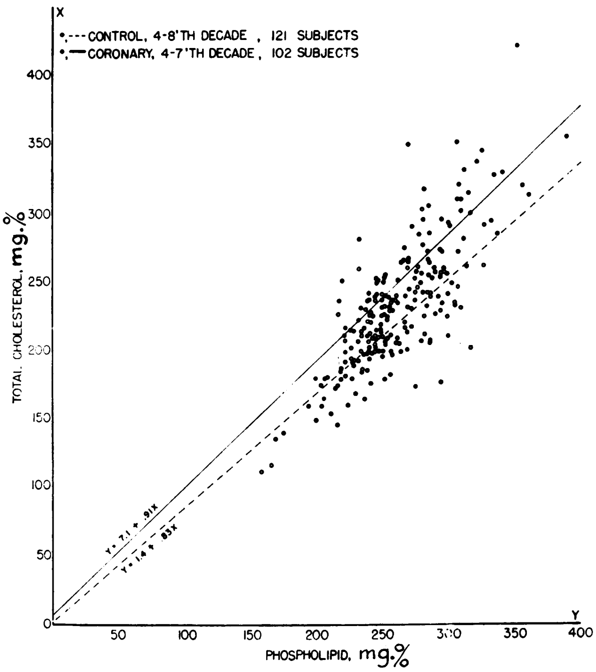

Fig. 4.—The relationship between total serum cholesterol and phospholipid.

this, the C/P ratios were carefully examined in the present study. We have previously reported[5] on the study of the C/P ratio in the healthy control group.

The close relationship between the concentrations of serum cholesterol and serum phospholipid is well known. Since, as described above, the correlation between free and total serum cholesterol is very high, either total or free cholesterol may be used as the numerator in the so-called C/P ratio. Jackson and Wilkinson[28] recommended the use of free cholesterol because its close relationship to phospholipids is maintained even in the presence of liver disease. However, in our subjects there was no liver disease, and total cholesterol was preferred as it has been more widely studied.

The effect of the fasting state on the C/P ratio was investigated. Serum cholesterol and phospholipid concentrations were determined in 23 coronary patients while fasting and again five hours after a fat-containing breakfast (bacon, two eggs, buttered toast, coffee with cream). The results were as follows:

	Mean (S.E.)		
	Fasting	*5 hours p.c.*	*p*
Total cholesterol..	270.4 (9.8)	280.8 (13.8)	>0.05
Phospholipid.....	264.9 (9.8)	294.0 (10.4)	<0.05

It is seen that the concentration of serum phospholopid, but not of cholesterol, is significantly elevated following a fatty meal. The relationship between serum cholesterol and phospholipid must, therefore, be studied in the fasting state. Not all previous workers have fulfilled this requirement.[17, 26, 29]

*Control subjects in the eighth decade are included in some of the work in this section.

TABLE V.—C/P RATIO§, MEAN AND STANDARD DEVIATION

	Decade			
Group	*4*	*5*	*6*	*7*
Control	.845 ±.062	.842 ±.101	.848 ±.076	.829 ±.068
Coronary	.982*** ±.097	.931*** ±.083	.909* ±.107	.907** ±.032

Significance of difference from the control group: *p <0.05; **p <0.01; ***p <0.001

§C/P = total serum cholesterol (mg.%) ÷ phospholipid (mg.%).

In Table V, the mean C/P ratio appears to be significantly higher in the coronary group. This might suggest an abnormal relationship between total serum cholesterol and phospholipid in C.H.D. However, Fig. 4 shows that the plot between the two lipids is linear in the coronary as well as in the control group. Application of the regression equation in Table VI demonstrates that in each decade the slope and intercept of these lines are not significantly different for the two groups. Furthermore, the correlation coefficients are similar. The averaged data for all decades of each group in Table VI also show no significant difference in the correlation coefficients or slopes. These data then clearly indicate that the relation between serum cholesterol and phospholipid is *not* disturbed in C.H.D.

In healthy men, Adlersberg and associates[29] found that C/P ratio increased with higher serum cholesterol values. To us it seemed possible that the association between C/P ratio and total serum cholesterol was spurious in nature,[30] arising from the imperfect correlation between serum cholesterol and phospholipid, so that high C/P ratios tend to be associated with high serum cholesterol values.

This was shown to be the case in our study. In Fig. 4 the imperfect correlation between total cholesterol and phospholipid results in a scattering of points above and below the regression line. The points above the line are associated with higher total serum cholesterol values and with higher C/P ratios than those below the line. In Table VII, the correlation coefficient between C/P ratio and total cholesterol is shown to be similar to what is expected from this less than perfect correlation between cholesterol and phospholipid[30] in both groups. Again the C/P ratio is similar in C.H.D. and in health.

TABLE VI.—THE RELATIONSHIP BETWEEN TOTAL SERUM CHOLESTEROL AND PHOSPHOLIPID SHOWN BY THE CORRELATION COEFFICIENT (r) AND THE SLOPE (b) AND INTERCEPT (a) OF THE REGRESSION EQUATION

Decade	*r*	*b ± s.e.*	**a ± s.e.*
Fourth			
Control	.85	.83 ± .10	3.7 ± 15.5
Coronary	.83	.92 ± .13	20.7 ± 22.0
Fifth			
Control	.73	.86 ± .17	3.3 ± 16.2
Coronary	.69	.87 ± .19	7.4 ± 21.7
Sixth			
Control	.74	.74 ± .14	3.6 ± 15.3
Coronary	.77	.99 ± .16	.8 ± 21.2
Seventh			
Control	.88	.88 ± .10	—.1 ± 16.6
Coronary	.72	.78 ± .16	—.7 ± 20.0
Eighth			
Control	.73	.83 ± .18	1.5 ± 16.3
Coronary	—	—	—
†Control (4-8th)	.79	.83 ± .06	1.4
‡Coronary (4-7th)	.77	.91 ± .08	—

*"a" is based on average "b".
†Pooled data for 121 subjects.
‡Averaged data for 102 subjects.

TABLE VII.—CORRELATION COEFFICIENT "r" BETWEEN C/P RATIO AND TOTAL SERUM CHOLESTEROL

	n	*r*	*Expected "r"†*
Control	121	0.63	0.60
Coronary	102	0.59*	0.62

*Average value of the four decades.
†Expected from the observed correlations between total serum cholesterol and phospholipid.

As to the discriminating ability of the C/P ratio for coronary and control subjects, it is shown below to be no better than total cholesterol and other lipid fractions. Therefore its use in the study of C.H.D. should be abandoned.

Lipoproteins With Cholesterol and With Phospholipid

Chemical analyses of the beta lipoproteins[31-33] show that the denser Std. S_f 0-12 fraction contains a much larger percentage of cholesterol and an equal or slightly larger percentage of phospholipid than the less dense 12-400 fractions. In the control group[5] the statistical correlations of the lipoprotein fractions with cholesterol and with phospholipid were in accord with these chemical findings.

TABLE VIII.
CORRELATION COEFFICIENTS OF LIPOPROTEIN FRACTIONS WITH TOTAL CHOLESTEROL AND WITH PHOSPHOLIPID

	Lipoprotein fraction	*r, Cholesterol**	*r, Phospholipid†*
	Std. S_f		
1.	0-12	.71	.53
2.	12-20	.58	.51
3.	20-100	.34	.52
4.	100-400	.23	.46
	0-400	.71	.73

*p <0.01 for differences between r 1 and 3, 1 and 4, 2 and 3, 2 and 4.
*p >0.05 for differences between r 1 and 2, 3 and 4.
‡p >0.05 for differences between all pairs of r.

The correlations of the lipoproteins with cholesterol and with phospholipid were determined in each decade of the coronary group. Since there were no significant differences between decades, the data for the entire group were pooled and are presented in Table VIII. The correlation with cholesterol decreases progressively from Std. S_f

0-12 to Std. S_f 100-400, as shown by the significant differences between the correlation coefficients in the subscript to this table. It is of interest that the correlation coefficient between cholesterol and lipoprotein fraction 10-20 was about 0.6, very similar to that reported earlier by Keys[34] in his re-analysis of Gofman's data[35] and by DeWind, Michaels and Kinsell.[36] The correlation with phospholipid is significant and similar for each lipoprotein fraction. Thus, the relation of cholesterol and phospholipid to the lipoproteins is the same in the coronary group as in the control group.[5]

The progressively changing correlation between the beta lipoprotein sub-fractions and cholesterol suggests a progressively increasing concentration of cholesterol as the density of beta lipoprotein increases. Previous chemical studies of beta lipoproteins have been performed on only two sub-fractions divided at approximately S_f 12. The present results suggest that chemical analysis of smaller beta lipoprotein sub-fractions should be done.

SEPARATION OF CORONARY AND CONTROL SUBJECTS BY SERUM LIPIDS AND LIPOPROTEINS

In the past decade it has become generally accepted that in groups of patients who have had myocardial infarctions, significant elevations of the serum lipids and lipoproteins can be demonstrated. However, there has been considerable—and passionate—disagreement as to the relative merits of the different serum lipids in distinguishing between coronary and non-coronary subjects. In 1950, Gofman *et al.*[37] developed the flotation technique for analytical ultracentrifugation of serum lipoproteins. The lipoproteins originally incriminated in C.H.D. were those in the S_f 12-20 class, and their correlation with clinical atherosclerosis was claimed to be better than that of cholesterol.[38] However, Keys,[34] after analyzing Gofman's data, denied this and indeed suggested that the ability of this lipoprotein fraction to discriminate between coronary and non-coronary persons might be due to its being (imperfectly) correlated with cholesterol. Gofman's group later devised an "atherogenic index"[39] by crediting certain lipoprotein fractions with more importance than others. This was subsequently modified into the "alpha value"[40] and, by incorporating the age factor, an "accumulated coronary disease value".[41]

Paterson *et al.*[20, 42] found no relation between serum cholesterol, S_f 12-20 and S_f 20-100 lipoproteins, the "alpha index" and the "alpha value" estimated serially during life, and the severity of coronary atherosclerosis found at autopsy. However, most of the patients examined were over the age of 60, when hyperlipidemia is infrequent.

The American co-operative prospective study[43] ended in a difference of opinion. Gofman's minority group modified the original method of lipoprotein determination to include additional classes and to correct for the effects of concentration on flotation rate. They concluded that the Std. S_f 12-20 lipoproteins and the "Atherogenic Index" measurements were superior to cholesterol in predicting definite new coronary events. However, the majority disagreed and held that the elevation of serum lipoprotein and cholesterol was not of clinical value in predicting which individuals would develop C.H.D. Since the cholesterol measurement was at least as useful as lipoprotein estimation, it was preferable in a practical sense because it was much easier and cheaper to perform. Lawry *et al.*[15] and the Albany group[44] found that although the mean serum cholesterol and lipoprotein levels were higher in C.H.D. than in health, the small size and great variability of the difference prevented the clinical prediction of C.H.D. among individuals. In diabetics who developed cardiovascular complications,[45] the predictive value of lipoproteins was no better than cholesterol; and both were applicable to groups but not to individuals. In a small series, Milch's group[46] preferred the lipoproteins as discriminators. In a larger study, Mattingly *et al.*[47] found that the elevation of serum cholesterol was greater and more distinctive than that of the S_f 12-20 and 20-100 lipoproteins. Rivin, Wong and Yoshino[48] measured total cholesterol, total stainable lipid, and ratio of alpha/beta stainable lipid in about 100 male survivors of myocardial infarction and 100 controls. Below the age of 40 years, all of these measurements partially discriminated the normal group from the coronary group. Above this age, the alpha/beta ratio provided the best segregation. In all studies of coronary and non-coronary groups, there has been considerable overlapping of the distributions of the serum lipids and lipoproteins.

In the present study, it is evident from Table IX that total serum cholesterol, ester cholesterol, free cholesterol, C/P ratio, Std. S_f 0-12 and 0-400 lipoproteins all have approximately similar "t" values and therefore similar abilities to separate coronary and control subjects. The "t" values for the remaining lipid fractions are relatively smaller. Log transformations of the data did not significantly change the results.

That the cholesterol ester and free fractions could not be better than total cholesterol in segregating the two groups must follow from the narrow range of per cent free to total cholesterol (Table III) found in C.H.D. as well as in health. A previous claim of the superiority of cholesterol ester[17] can be attributed to faulty laboratory technique as indicated by the grossly abnormal per cent of free cholesterol. It is of interest that the lipoproteins exhibited no advantages and that Std. S_f 12-20 lipoproteins, originally suggested as the superior discriminator,[38] were the least impressive, achieving significance only in the fourth decade.

TABLE IX.—"t" TEST BETWEEN THE CONTROL AND CORONARY GROUPS FOR EACH SERUM LIPID FRACTION

Decade	*Fourth*		*Fifth*		*Sixth*		*Seventh*	
	t	*p*	*t*	*p*	*t*	*p*	*t*	*p*
Total cholesterol.	6.15	<.001	4.78	<.001	4.07	<.001	1.46	>.1
Ester cholesterol.	6.07	<.001	4.55	<.001	3.78	<.001	1.53	>.1
Free cholesterol.	5.84	<.001	4.89	<.001	4.59	<.001	1.14	>.1
C/P ratio....	5.95	<.001	3.44	<.001	2.42	<.05	3.12	<.01
Std. S_f 0-12..	5.23	<.001	4.69	<.001	3.70	<.001	2.83	<.01
Std. S_f 0-400.	5.37	<.001	5.34	<.001	4.00	<.001	0.72	>.1
Phospholipid.	3.73	<.001	3.11	<.01	3.33	<.001	—	—
Std. S_f 12-20.	2.95	<.01	1.69	>.05	—	—	—	—
Std. S_f 20-100	2.55	<.05	2.69	<.01	2.72	<.01	—	—
Std. S_f 100-400....	2.03	<.05	1.81	>.05	4.59	<.001	—	—
Std. S_f 12-400	2.97	<.01	2.68	<.01	3.20	<.01	—	—

— = control mean greater than coronary mean.

Another method of assessing distinguishing ability is by "per cent misclassification". The figure half way between the mean lipid levels of the two groups is obtained and the number of individuals whose values place them on the wrong side of this figure is expressed as a percentage of the total number of men. The results are summarized in Table X. Again, total serum cholesterol, ester cholesterol, free cholesterol, C/P ratio, Std. S_f 0-12 and 0-400 lipoproteins all have approximately equal ability to distinguish between control and coronary individuals. The remaining lipid fractions in the bottom half of the table give considerably higher per cent misclassifications. Table X also shows that the distinguishing ability of any serum lipid is highest in the fourth and fifth decades and at best has a 20% error. In the older decades increasing numbers are misclassified (approximately 33% in the seventh).

TABLE X.—PER CENT MISCLASSIFICATION OF CONTROL AND CORONARY SUBJECTS BY EACH SERUM LIPID FRACTION

Decade	*Fourth*	*Fifth*	*Sixth*	*Seventh*	*Average*
Total cholesterol...	22%	24%	27%	39%	28.0%
Ester cholesterol...	18	24	33	41	28.9
Free cholesterol	22	22	25	39	27.0
C/P ratio.....	16	31	36	28	28.0
Std. S_f 0-12....	18	22	29	37	26.3
Std. S_f 0-400...	20	24	33	39	28.8
Std. S_f 12-20...	28	41	42	—	—
Std. S_f 20-100..	30	37	34	—	—
Std. S_f 100-400.	46	35	25	—	—
Std. S_f 12-400..	36	37	33	—	—
Phospholipid...	26	31	31	—	—
% free........	36	25	40	—	—

— = control mean greater than coronary mean.

DISCRIMINANT ANALYSIS BETWEEN CONTROL AND CORONARY SUBJECTS COMBINING TOTAL SERUM CHOLESTEROL, PHOSPHOLIPID AND THE STD. S_f 0-12, 12-20, 20-100 AND 100-400 LIPOPROTEINS

Discriminant analysis was carried out in an attempt to separate all the subjects into coronary and control groups solely on the basis of their serum lipid and lipoprotein levels. The F values in Table XI show significant discrimination by these combined serum lipid fractions in all decades, even in the seventh. The per cent misclassification of the control and coronary subjects, by using the distributions calculated from the discriminant functions, is also given in the final column of Table XI. In each decade, the per cent misclassification tends to be smaller than that for the indi-

TABLE XII.—MISCLASSIFICATION OF CONTROL AND CORONARY SUBJECTS CALCULATED FROM THE DISCRIMINANT FUNCTIONS OF THE SERUM LIPIDS AND LIPOPROTEINS

Decade	*No. of subjects total*	*No. of variables**	*No. of subjects misclassified*
4	50	1	11
		2	8
		3	9
		4	8
		5	8
		6	6
5	51	1	12
		2	12
		3	11
		4	11
		5	11
		6	9
6	55	1	15
		2	17
		3	13
		4	14
		5	11
		6	11
7	46	1	18
		2	17
		3	15
		4	13
		5	13
		6	13

*Variables.

1. Total cholesterol.
2. Total cholesterol + (0-12).
3. Total cholesterol + (0-12) + (12-20).
4. Total cholesterol + (0-12) + (12-20) + (20-100).
5. Total cholesterol + (0-12) + (12-20) + (20-100) + (100-400).
6. Total cholesterol + (0-12) + (12-20) + (20-100) + (100-400) + phospholipid.

TABLE XI.—DISCRIMINANT ANALYSIS AND PER CENT MISCLASSIFICATION BETWEEN CONTROL AND CORONARY SUBJECTS USING ALL THE SERUM LIPID FRACTIONS*

	Discriminant function coefficients							
Decade	*T. cholesterol*	*Phospho-lipid*	*Std S_f 0 - 12*	*Std S_f 12 - 20*	*Std S_f 20 - 100*	*Std S_f 100 - 400*	*F*	*% misclassification*
4	−.00157	.00111	−.00008	.00038	−.00027	−.00020	7.76 p <.01	12
5	−.00043	.00041	−.00032	.00003	−.00011	−.00029	5.40 p <.01	18
6	−.00036	.000003	−.00025	.00064	−.00020	−.00093	5.83 p <.01	20
7	−.00087	.00095	−.00041	.00076	.00012	−.00021	2.95 p <.05	28

*Log transformation of the data made no significant differences in the results.

vidual serum lipid values previously shown in Table X. Also in each decade, as shown in Table XII, there is a tendency toward progressively improved discrimination with the addition of each serum lipid fraction to the analysis. The data from Tables X, XI and XII indicate that by the use of all the serum lipid fractions, there is some improvement in discrimination between control and coronary subjects, but this improvement over the discrimination by total serum cholesterol alone is not sufficient to offer clinical utility. This is not to say that other serum lipid fractions as yet unmeasured, such as the phospholipid sub-fractions, may not have more practical diagnostic usefulness.

TRIGLYCERIDES AND THE SERUM LIPIDS

Albrink, Meigs and Man[50, 51] claimed that fasting serum triglycerides or the triglyceride-containing lipoprotein fractions correlate better with clinical atherosclerosis than does cholesterol or other serum lipids. The evidence on which these conclusions are based is unconvincing.[49-53] In one widely quoted study[50] there were changing laboratory methods and incomplete presentation and analysis of data. Although in the present study serum triglycerides are not determined as such, the 100-400 Std. S_f lipoproteins provide some measure of this fraction, since they comprise a large portion of the total serum triglyceride. It is obvious from Tables IX and X that Std. S_f 100-400 lipoproteins are not as highly correlated with clinical C.H.D. as is total cholesterol.

In Table XIII, the per cent misclassification of coronary and control subjects is determined from triglyceride and lipoprotein data reported in the literature. The serum triglycerides reported by Albrink[51] and the Std. S_f 12-400 lipoproteins (containing a large portion of the triglyceride), reported by Gofman *et al.*,[49] provide roughly the same misclassification of subjects as cholesterol, phospholipid and Std. S_f 0-12 lipoprotein in the present study. It seems likely, therefore, that no one of these fractions has any superior discriminating ability over the other. Obviously, none of them can be considered prognostic or diagnostic for C.H.D. in the individual subject. We agree with a recent editorial opinion[54] that the relative merits of serum cholesterol and serum triglycerides in predicting C.H.D. require additional study. Furthermore, we agree that for an individual patient it is desirable to estimate both total cholesterol and serum triglyceride, since one or other or both fractions may be elevated in various lipid metabolic defects predisposing to atherosclerosis.

It is concluded that serum lipid measurements can discriminate between groups of coronary and non-coronary subjects, but the separation is not sufficiently precise to be diagnostic for the individual. However, in context with the clinical history and findings, the concentrations of the serum lipids are of practical usefulness, especially under age 60. For this purpose, estimation of total serum cholesterol and triglyceride gives a reasonably complete picture of the serum lipids. Finally, it must be recognized that although serum lipoproteins do not appear to be superior to total cholesterol in characterizing coronary groups, the study of lipoproteins is of fundamental importance in understanding lipid metabolism and transport, as emphasized by Gofman and his group.

TABLE XIII.
PER CENT MISCLASSIFICATION IN CORONARY AND CONTROL SUBJECTS FROM TWO STUDIES IN THE LITERATURE

	Decade				
Serum fraction, author	*4*	*5*	*6*	*7*	*8*
Triglyceride, Albrink[52]*..	19%	35%	28%	30%	10%†
Std. S_f 12-400 lipoprotein, Gofman *et al.*[49]‡......		33%	36%	46%	

*% misclassification calculated around a value half way between the medians in Fig. 1.[52]
†Only six subjects were present in the coronary group.
‡Calculated from the standard scores.[49]

CORRELATION OF TOTAL SERUM CHOLESTEROL TO BODY MEASUREMENTS

The reliability of the early investigations of the correlation of lipid levels with body build was limited by the difficulty of physique classification. More recently Gertler and White,[18] employing the Sheldon system of somatotyping in their young coronary patients, found the serum cholesterol to be somewhat higher in the mesomorph (the squared, muscular type). Lawry *et al.*[15] concluded that the trend of serum lipid levels to rise with age was partly attributable to fattening with age.

In this study an attempt was made to determine whether, in coronary heart disease, the body measurements taken in "routine" clinical practice could be related to the level of serum cholesterol. The following measurements were recorded on all subjects: (1) height (without shoes, to the nearest half-inch), (2) chest circumference (midsternal level, midway between inspiration and expiration, to the nearest half-inch), (3) weight (in underclothes, to the nearest half-pound). A *height-weight* index and *body build* index were calculated as previously described.[5]

The data for these body measurements have been presented previously.[1] In each decade, the average height of the coronary patients was approximately 67.3 (S.E., .5) inches and the average chest circumference approximately 38.0 (S.E., .4) inches. The average weight was 167 pounds in the fourth decade, decreasing progressively to 156 in the seventh (S.E. approximately 4.0). It must be emphasized that the variations within groups are small.

For each decade, correlation coefficients were computed between total serum cholesterol on the one hand and the three body measurements and two body indices on the other. None was significant.

In this coronary group, as in the healthy group,[5] there was no demonstrable relation of total serum cholesterol to body height, chest circumference, weight, *height-weight* index or *body build* index.

CORRELATION OF TOTAL SERUM CHOLESTEROL TO PHYSICAL ACTIVITY

The traditional view, based on little scientific evidence, has been that physical activity, by increasing "wear and tear", was at least an aggravating factor in coronary atherosclerosis. Recently,[55, 56] C.H.D. has been found to more prevalent in the sedentary than among those actively engaged. In our study, however, C.H.D. was associated with habitually greater physical activity.[1]

The degree of activity (minimal, moderate, most) at the time of investigation was assessed in the coronary patients. An analysis of variance demonstrated no significant association with the level of serum total cholesterol in any decade.

The practice of noting the subject's last occupation rather than his usual one may lead to fallacious conclusions. This is especially true in C.H.D., where many patients have their activity restricted. Accordingly, "life-long" activity of the patients was considered and classified. Again, there was no association between serum cholesterol and degree of physical activity. In this sample of C.H.D. patients then, as in the healthy group,[5] there was no demonstrable relationship between the level of total serum cholesterol and physical activity, either present or life-long. The possibilities remain that greater amounts of exercise than were found in this group may lower fasting lipids, or that exercise lowers the postprandial serum lipid peaks.

CORRELATION OF TOTAL SERUM CHOLESTEROL AND FAMILY HISTORY OF C.H.D.

An attempt was made to determine whether the familial tendency to C.H.D. could be associated with the level of serum cholesterol. A detailed family history with particular reference to presence of C.H.D. was obtained by direct questioning of each coronary patient. In instances where information was of doubtful validity no record was made. The data for the incidence of C.H.D. in aunts and uncles were too unreliable, and those for the siblings, grandparents and mothers too few in number, to warrant analysis. Table XIV contrasts the cholesterol levels of the coronary patients with and without fathers who had C.H.D. There were no significant differences.

TABLE XIV.—SERUM TOTAL CHOLESTEROL (MEAN, mg.%) RELATED TO HISTORY OF C.H.D. IN FATHERS OF CORONARY PATIENTS

		Positive		*Negative*		*Differences between*
Decade	*n*	*n*	*Cholesterol*	*n*	*Cholesterol*	*means*
Fourth....	25	12	281.4	10	281.7	n.s.
Fifth.....	26	11	264.3	13	259.6	n.s.
Sixth.....	28	6	229.8	18	260.5	n.s.
Seventh...	23	2	223.0	17	227.9	n.s.

n.s. = not significant.

C.H.D. is often familial and often associated with hypercholesterolemic states; the level of the serum lipids is genetically influenced. It was expected, therefore, that higher serum cholesterol levels would be demonstrated in patients with a positive family history. The failure to do so may be due to the fact that the mechanisms of the familial occurrence of C.H.D. may be so varied[1] that it would require much larger numbers to show a relationship to serum lipid values clearly.

SUMMARY AND CONCLUSIONS

Serum total, free and ester cholesterol, phospholipid, C/P ratio and standard (Std.) S_f 0-400 lipoprotein fractions were determined in 102 male survivors of myocardial infarction evenly distributed from the fourth to the seventh decades. All had atherosclerosis of the "essential" type, that is, not accompanied by high blood pressure or any disease associated with secondary hypercholesterolemia. A hundred men from the same general population, found to be clinically healthy, were matched by age as controls.

In the coronary group, the distributions of all the lipid fractions were lognormal. All the serum lipids were significantly elevated above normal. The maximum elevations occurred in the fourth decade; the concentration of all the fractions then progressively decreased to the seventh decade, where they no longer differed significantly from the controls. The decrease in serum cholesterol with age was 15.4 mg. % per decade. This decrease may be attributed to three facts: (1) The higher the serum cholesterol level, the younger the age of onset of clinical coronary heart disease (C.H.D.). (2) Subsequent to the onset of C.H.D., the high mortality, which is nearly equal in each decade, means that most coronary patients, including the young ones with high cholesterol levels, die before entering the next decade. (3) In individual survivors of a myocardial infarct repeated determinations of serum cholesterol over a five- to seven-year period showed an average decline in serum cholesterol of 1.3 mg. % per year.

As in health, the percentage free to total serum cholesterol was a variable with a small dispersion from the mean of 28.

As in health, the imperfect correlation between total serum cholesterol and phospholipid produced a

spurious correlation between the C/P ratio and total serum cholesterol. Since serum phospholipid but not cholesterol rises significantly following a fatty meal, the C/P ratio must be studied in the postabsorptive state.

As in health, the correlation of serum cholesterol with lipoproteins was greatest for the Std. S_f 0-12 fraction (r = .71) and progressively decreased for the other fractions, Std. S_f 12-20, 20-100 and 100-400.

As in health, the correlation of phospholipid with the lipoproteins was similar for each fraction (approximately .5).

In C.H.D., then, there is no abnormality in the relationship of the various cholesterol, phospholipid and lipoprotein fractions to one another.

Total serum cholesterol and Std. S_f 0-12 and 0-400 lipoproteins have approximately equal ability to separate coronary and non-coronary subjects. The separation is best in the fourth decade (20% misclassification) and decreases progressively to the seventh (33% misclassification). The C/P ratio provides no improvement in discrimination and its use should be abandoned.

The discriminating ability of the triglyceride-containing Std. S_f 100-400 lipoprotein fraction was not nearly so good as that of total cholesterol or as that of the cholesterol-containing Std. S_f 0-12.

Using all the lipid and lipoprotein fractions in a discriminant analysis improved the separation of these coronary and non-coronary subjects. In the fourth decade the misclassification was only 12%.

Because of comparative accuracy and cheapness, the determination of total serum cholesterol remains the single best *screening* test for the presence of hyperlipidemia. When combined with estimation of triglycerides it is probable that most of the important serum lipid disturbances will be detected. Quantitative lipoprotein fractionation, while relatively impractical, remains an important research method.

In the coronary group there was no demonstrable relationship of total serum cholesterol to body measurements (weight, height, chest circumference, *height-weight index, body build index*) to physical activity (past or present) or to family history of C.H.D. However, the variations of these factors within the group were relatively small.

Finally, it should be cautioned that the patients in this retrospective study were highly selected ("essential" atherosclerosis, equal numbers in each decade, etc.). The results, therefore, while showing the relationship of serum lipids to C.H.D. in the absence of other variables, need not necessarily apply to the general coronary population in every respect.

The continued support of Professor R. Ian Macdonald is gratefully acknowledged. The biochemical determinations were done by Mrs. C. Radecka, M.A.; the lipoproteins by the University of McGill Ultracentrifuge Laboratory under the direction of Dr. R. F. Robertson; Dr. E. A. Murphy, Professor D. B. W. Reid and Mrs. A. Csima contributed to the statistical analysis. We are sincerely thankful to our excellent secretary, Miss Ruth Yano.

REFERENCES

1. SHANOFF, H. M. *et al.*: *Canad. Med. Ass. J.*, **84**: 519, 1961.
2. LITTLE, J. A. *et al.*: *Circulation*, **10**: 585, 1954 (abstract).
3. *Idem*: *Ibid.*, **14**: 500, 1956 (abstract).
4. LITTLE, J. A. AND SHANOFF, H. M.: *Ibid.*, **16**: 482, 1957 (abstract).
5. LITTLE, J. A., VAN DER FLIER, R. W. AND SHANOFF, H. M.: *Canad. Med. Ass. J.*, **86**: 1045, 1962.
6. BIÖRCK, G., BLOMQVIST, G. AND SIEVERS, J.: *Acta Med. Scand.*, **156**: 493, 1957.
7. DODDS, C. AND MILLS, G. L.: *Lancet*, **1**: 1160, 1959.
8. SPERRY, W. M. AND WEBB, M.: *J. Biol. Chem.*, **187**: 97, 1950.
9. ZILVERSMIT, D. B. AND DAVIS, A. K.: *J. Lab. Clin. Med.*, **35**: 155, 1950.
10. DE LALLA, O. F. AND GOFMAN, J. W.: Ultracentrifugal analysis of serum lipoproteins. *In*: Methods of biochemical analysis, vol. 1, edited by D. Glick, Interscience Publishers, Inc., New York, 1954, p. 459.
11. CASSIDY, M.: *Lancet*, **2**: 587, 1946.
12. LITTLE, J. A.: *Canad. Med. Ass. J.*, **83**: 1202, 1960.
13. DAVIS, D., STERN, B. AND LESNICK, G.: *Ann. Intern. Med.*, **11**: 354, 1937.
14. OLIVER, M. F. AND BOYD, G. S.: *Brit. Heart J.*, **15**: 387, 1953.
15. LAWRY, E. Y. *et al.*: *Amer. J. Med.*, **22**: 605, 1957.
16. HUNTER, J. D.: *New Zeal. Med. J.*, **60**: 235, 1961.
17. GERTLER, M. M., GARN, S. M. AND LERMAN, J.: *Circulation*, **2**: 205, 1950.
18. GERTLER, M. M. AND WHITE, P. D.: Coronary heart disease in young adults, Harvard University Press, Cambridge, Mass., 1954.
19. WHITE, N. K., EDWARDS, J. E. AND DRY, T. J.: *Circulation*, **1**: 645, 1950.
20. PATERSON, J. C., DYER, L. AND ARMSTRONG, E. C.: *Canad. Med. Ass. J.*, **82**: 6, 1960.
21. OLIVER, M. F.: *Scot. Med. J.*, **3**: 225, 1958.
22. HOOD, B. AND ANGERVALL, G.: *Amer. J. Med.*, **26**: 30, 1959.
23. ROE, R. D., LITTLE, A. AND SHANOFF, H. M.: *Circulation*, **22**: 800, 1960 (abstract).
24. LITTLE, J. A. AND SHANOFF, H. M.: Unpublished observations.
25. PETERS, J. P. AND VAN SLYKE, D. D.: Quantitative clinical chemistry, vol. 1, 2nd ed., Williams and Wilkins Conmpany, Baltimore, 1946, p. 472.
26. STEINER, A., KENDALL, F. E. AND MATHERS, J. A. L.: *Circulation*, **5**: 605, 1952.
27. MORRISON, L. M., GONZALEZ, P. AND WOLFSON, E.: *Ibid.*, **2**: 472, 1950.
28. JACKSON, R. S. AND WILKINSON, C. F., JR.: *J. Amer. Geriat. Soc.*, **1**: 447, 1953.
29. ADLERSBERG, D. *et al.*: *J. A. M. A.*, **162**: 619, 1956.
30. PEARSON, K.: *Proc. Roy. Soc.*, **60**: 489, 1897.
31. LINDGREN, F. T., NICHOLS, A. V. AND FREEMAN, N. K.: *Journal of Physical Chemistry*, **59**: 930, 1955.
32. HAVEL, R. J., EDER, H. A. AND BRAGDON, J. H.: *J. Clin. Invest.*, **34**: 1345, 1955.
33. CORNWELL, D. G. AND KRUGER, F. A.: *J. Lipid Res.*, **2**: 110, 1961.
34. KEYS, A.: *J. A. M. A.*, **147**: 1514, 1951.
35. GOFMAN, J. W. *et al.*: *Circulation*, **2**: 161, 1950.
36. DEWIND, L. T., MICHAELS, G. D. AND KINSELL, L. W.: *Ann. Intern. Med.*, **37**: 344, 1952.
37. GOFMAN, J. W. *et al.*: *Science*, **111**: 166, 1950.
38. JONES, H. B. *et al.*: *Amer. J. Med.*, **11**: 358, 1951.
39. GOFMAN, J. W. *et al.*: *Mod. Med. (Minneap.)*, June 15, 1953, p. 119.
40. GOFMAN, J. W.: *Industr. Med. Surg.*, **24**: 151, 1955.
41. LYON, T. P. *et al.*: *Calif. Med.*, **84**: 325, 1956.
42. PATERSON, J. C., CORNISH, B. R. AND ARMSTRONG, E. C.: *Circulation*, **13**: 224, 1956.
43. Committee on Lipoproteins and Atherosclerosis, Technical Group, and National Advisory Heart Council, Committee on Lipoproteins and Atherosclerosis: *Ibid.*, **14**: 691, 1956, Part 2.
44. DOYLE, J. T. *et al.*: *J. Chronic Dis.*, **6**: 33, 1957.
45. LOWY, A. D., JR. AND BARACH, J. H.: *Circulation*, **17**: 14, 1958.
46. SCHLESSINGER, B. S. *et al.*: *Ibid.*, **19**: 265, 1959.
47. MATTINGLY, T. W. *et al.*: *J. A. M. A.*, **170**: 536, 1959.
48. RIVIN, A. U., WONG, H. AND YOSHINO, J.: *Amer. J. Cardiol.*, **5**: 784, 1960.
49. GOFMAN, J. W. *et al.*: *Physiol. Rev.*, **34**: 589, 1954.
50. ALBRINK, M. J., MEIGS, J. W. AND MAN, E. B.: *Amer. J. Med.*, **31**: 4, 1961.
51. ALBRINK, M. J.: *Arch. Intern. Med. (Chicago)*, **109**: 345, 1962.
52. CARLSON, L. A.: *Acta Med. Scand.*, **167**: 377, 1960.
53. SCHRADE, W., BOEHLE, E. AND BIEGLER, R.: *Lancet*, **2**: 1409, 1960.
54. HAVEL, R. J. AND CARLSON, L. A.: *Metabolism*, **11**: 195, 1962.
55. MORRIS, J. N. *et al.*: *Lancet*, **2**: 1053, 1111, 1953.
56. BENTON, J. G. AND RUSK, H. A.: *Ann. Intern. Med.*, **41**: 910, 1954.

APPENDIX—SERUM LIPID FRACTIONATION (mg.%) IN CANADIAN MALES WITH C.H.D.

Number	Study No.	Age	Cholesterol				Phospholipid	C/P ratio	STD. S_f lipoproteins				
			Total	Ester	Free	% free			0-12	12-20	20-100	100-400	0-400
					Fourth decade								
1	11	38	328	231	97	29.6	342	0.959	511	84	94	17	706
2	38	35	265	177	88	33.2	284	0.933	177	101	285	88	651
3	51	30	235	172	62	26.6	217	1.083	344	38	51	14	447
4	61	37	348	252	96	27.2	369	0.943	417	177	168	84	846
5	86	39	298	213	85	28.6	315	0.946	391	98	116	46	651
6	89	33	239	169	70	29.4	244	0.979	398	83	104	51	636
7	92	38	350	249	101	28.9	306	1.142	587	53	68	30	738
8	93	39	354	242	112	31.8	389	0.910	568	88	240	271	1167
9	95	33	283	203	80	28.3	277	1.020	499	101	133	83	816
10	99	39	253	182	71	28.0	293	0.864	383	69	133	110	695
11	100	34	271	197	75	27.7	283	0.957	404	80	189	104	777
12	104	39	230	165	65	28.3	233	0.986	391	60	71	28	550
13	107	35	222	158	64	28.6	232	0.956	275	54	73	62	464
14	112	39	300	213	87	29.0	308	0.975	473	88	159	78	798
15	113	38	252	184	68	27.0	245	1.029	392	75	118	38	623
16	115	39	421	293	128	31.2	352	1.169	774	119	104	24	1021
17	117	32	255	184	71	27.9	279	0.915	306	65	104	60	535
18	120	33	250	197	53	21.3	219	1.140	333	48	54	3	438
19	122	34	316	228	88	27.8	281	1.122	649	92	129	17	887
20	124	38	319	224	96	29.9	307	1.039	552	112	103	24	791
21	131	39	226	159	67	29.8	290	0.779	374	41	163	69	647
22	135	39	240	163	77	32.1	299	0.803	357	63	134	46	600
23	136	36	216	148	68	31.2	222	0.974	339	16	17	0	372
24	137	34	292	206	86	29.4	299	0.976	426	68	181	175	850
25	138	37	210	150	60	28.5	221	0.950	336	11	69	17	433
Geometric mean			274.4	195.2	78.9		280.4		408.4	66.0	106.9	37.8	660.1
95% limits			192.3 391.7	137.2 277.9	52.2 119.2		202.2 389.1		226.0 738.1	20.5 212.0	34.3 333.3	3.5 402.4	378.6 1150.9

APPENDIX—SERUM LIPID FRACTIONATION (mg.%) IN CANADIAN MALES WITH C.H.D.

Number	Study No.	Age	Cholesterol				Phospholipid	C/P ratio	STD. S_f lipoproteins				
			Total	Ester	Free	% free			0-12	12-20	20-100	100-400	0-400
					Fifth decade								
1	2	47	248	175	73	29.5	302	0.821	529	28	99	56	712
2	5	47	250	179	71	28.2	261	0.958	415	47	106	40	608
3	34	48	259	184	75	28.8	288	0.899	368	54	130	57	609
4	43	43	266	190	76	28.6	269	0.875	314	63	137	84	598
5	45	43	294	210	84	28.6	294	1.000	330	94	269	64	757
6	48	49	289	207	82	28.4	272	1.062	386	83	105	12	586
7	64	48	244	174	70	28.5	270	0.903	350	30	62	49	491
8	83	44	238	170	68	28.6	292	0.815	317	58	37	9	421
9	91	47	304	223	81	26.5	284	1.070	594	109	174	63	940
10	101	43	257	184	73	28.4	275	0.934	376	104	116	47	643
11	105	42	254	182	72	28.3	252	1.008	432	124	99	17	672
12	106	49	326	222	104	31.9	333	0.979	564	93	184	95	936
13	108	40	254	190	64	25.4	283	0.893	383	84	86	41	594
14	109	46	280	200	80	28.4	311	0.900	464	78	193	154	889
15	110	42	214	154	60	28.3	248	0.863	297	69	103	17	486
16	111	44	226	159	67	29.6	251	0.900	404	63	157	68	692
17	119	45	263	188	75	28.4	264	0.995	424	41	69	3	537
18	121	42	291	211	80	27.6	299	0.973	544	73	63	3	683
19	123	49	258	180	78	30.2	296	0.872	415	83	155	39	692
20	126	45	231	169	62	26.8	255	0.907	376	39	71	28	514
21	127	41	273	202	72	26.2	266	1.027	476	53	45	0	574
22	128	48	228	160	68	29.8	257	0.888	383	84	103	32	602
23	129	40	284	192	92	32.5	336	0.846	298	32	213	297	840
24	130	42	200	140	60	29.9	268	0.747	208	43	267	282	800
25	132	40	337	237	100	29.9	321	1.050	656	32	112	0	800
26	134	43	248	176	72	29.2	245	1.011	303	67	69	11	450
Geometric mean			260.3	185.5	74.6		279.4		395.5	61.2	109.9	27.8	643.8
95% limits			204.7 330.9	145.9 235.8	57.0 97.8		234.4 332.9		240.1 651.4	26.6 141.0	40.5 298.2	1.5 525.7	419.4 988.0

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APPENDIX—SERUM LIPID FRACTIONATION (mg.%) IN CANADIAN MALES WITH C.H.D.

Number	Study No.	Age	*Cholesterol* Total				Phospholipid	C/P ratio	STD. S_f lipoproteins 0-12				
			Total	*Ester*	*Free*	*% free*			*0-12*	*12-20*	*20-100*	*100-400*	*0-400*
					Sixth decade								
1	1	54	274	202	72	26.4	292	0.938	463	90	120	130	803
2	4	56	293	203	88	29.9	332	0.882	413	84	200	120	817
3	10	52	312	227	85	27.4	365	0.854	433	63	187	111	794
4	15	59	183	127	56	30.4	234	0.781	201	43	151	78	473
5	17	58	248	168	80	32.0	278	0.891	345	64	152	77	638
6	18	51	173	122	51	29.3	275	0.628	223	40	129	82	474
7	28	55	344	250	94	27.4	325	1.060	377	77	154	95	703
8	40	58	248	177	71	28.4	250	0.991	309	40	40	6	395
9	42	57	208	146	62	29.8	244	0.852	313	74	116	61	564
10	49	56	252	184	68	27.0	251	1.000	326	25	48	12	411
11	52	51	294	209	85	29.0	280	1.050	334	48	53	24	459
12	53	56	264	188	76	28.9	269	0.980	331	55	118	74	578
13	54	56	242	168	74	30.8	285	0.849	362	89	79	20	550
14	55	59	232	166	66	28.6	304	0.762	280	47	98	10	435
15	57	56	212	146	66	31.0	286	0.740	392	94	197	57	740
16	58	58	204	144	60	29.2	252	0.810	350	42	68	18	478
17	62	56	185	137	48	25.7	218	0.849	345	33	57	32	467
18	63	52	235	173	62	26.4	238	0.988	313	61	100	28	502
19	66	58	240	177	63	26.1	245	0.980	382	53	133	71	639
20	69	55	308	224	84	27.2	308	1.000	407	101	235	124	867
21	70	52	234	161	73	31.0	262	0.882	391	61	101	61	614
22	74	55	179	127	52	29.0	207	0.865	262	42	69	30	403
23	76	55	213	150	63	29.8	248	0.859	300	23	74	12	409
24	78	54	284	203	81	28.4	285	1.000	347	75	104	56	582
25	80	57	330	237	93	28.0	311	1.060	301	69	119	74	563
26	81	54	252	170	82	32.3	287	0.877	318	66	52	59	495
27	85	54	308	215	93	30.2	306	1.010	437	86	84	21	628
28	90	59	250	174	76	30.2	246	1.020	348	45	47	14	454
Geometric mean			245.8	174.4	71.1		272.1		337.3	56.4	98.7	41.1	553.9
95% limits			169.6	118.9	49.2		210.7		232.4	26.3	38.0	7.5	349.8
			356.1	255.9	102.7		351.5		489.6	120.8	256.4	225.8	877.0

APPENDIX—SERUM LIPID FRACTIONATION (mg.%) IN CANADIAN MALES WITH C.H.D.

Number	Study No.	Age	*Cholesterol* Total				Phospholipid	C/P ratio	STD. S_f lipoproteins 0-12				
			Total	*Ester*	*Free*	*% free*			*0-12*	*12-20*	*20-100*	*100-400*	*0-400*
					Seventh decade								
1	3	67	210	150	60	28.8	299	0.702	353	56	69	27	505
2	7	68	236	171	65	27.4	257	0.918	381	56	46	35	518
3	9	61	271	181	90	33.2	307	0.883	328	80	228	160	796
4	16	68	224	159	65	29.2	253	0.885	342	25	59	31	457
5	20	63	214	155	59	27.6	227	0.943	282	17	34	7	340
6	24	66	198	145	53	26.7	222	0.892	353	46	53	35	487
7	25	64	260	189	71	27.2	313	0.831	313	18	18	6	355
8	27	62	199	144	55	27.6	248	0.802	297	42	44	4	387
9	29	63	214	158	56	26.4	245	0.872	312	40	104	27	483
10	33	64	313	216	97	30.8	314	1.000	324	67	76	14	481
11	35	62	236	165	71	30.2	239	0.987	306	96	110	29	541
12	36	61	239	169	70	29.4	252	0.947	301	42	72	30	445
13	37	68	179	125	54	30.0	199	0.900	291	38	95	12	436
14	44	67	263	191	72	27.2	285	0.923	395	71	67	13	546
15	59	67	241	171	70	29.0	239	1.010	321	42	54	20	437
16	65	63	275	201	74	27.1	280	0.982	353	66	121	48	588
17	67	64	264	188	76	28.9	265	0.996	413	50	85	49	597
18	71	64	191	133	58	30.1	228	0.837	248	45	62	17	372
19	73	65	280	213	67	23.8	232	1.200	375	62	53	30	520
20	75	60	232	171	61	26.2	283	0.820	467	37	66	38	608
21	77	62	169	125	44	26.0	206	0.820	198	20	26	12	256
22	79	61	202	140	62	30.8	244	0.828	285	42	48	12	387
23	82	69	210	148	62	29.4	238	0.882	284	38	18	4	344
Geometric mean			228.6	163.6	64.8		253.4		322.2	43.5	59.5	19.9	460.2
95% limits			168.7	120.5	46.1		197.5		227.1	17.9	18.8	3.6	285.5
			309.9	222.2	91.0		325.3		457.2	105.9	188.2	109.3	741.9