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Interlipid Relationships in Clinical Atherosclerosis.* (28364)

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In view of the large amount of evidence that cholesterol plays an important role in atherosclerosis(1), it is surprising that serum cholesterol levels have little predictive value for the occurrence of myocardial infarction or other complications of coronary atherosclerosis(2). Also, it is surprising that the average cholesterol level of "atherosclerotic" persons is only about 10% higher than "normals," while individuals in either group vary over a range of 300%(3). Lacking, therefore, evidence clearly tying the absolute level of serum cholesterol to clinical atherosclerosis, we have considered the possibility that there might instead be some significant abnormality in its relative level.

This approach is suggested by the report of Peters and Man(4) that there is a great constancy in the relations between cholesterol and the phospholipids in a wide variety of ages and clinical states. Somewhat later, Popjak(5) reexamined these and other data, and in addition data from other species, and found that the curve relating phospholipids (P) to cholesterol (C) became linear when plotted logarithmically. He, therefore, fitted the equation $\text{Log } P = a + b \text{ Log } C$ by the method of least squares and found the constants essentially identical for all age groups, all clinical states and all animal species examined. He concluded that the mathematical relationship between serum cholesterol and phospholipids was a biological constant. Any significant disturbance in this fundamental relationship should, therefore, have substantial implications.

Neither Peters and Man nor Popjak examined data from patients with myocardial infarction or cerebral thrombosis, and we have accordingly subjected our data on such patients, as well as healthy and chronically ill control groups, to the statistical procedures used by Popjak. In confirmation of the prior reports, we find that the 2 control groups have essentially identical relationships between cholesterol and phospholipids, and that these relationships are not affected by age. However, the 2 atherosclerotic groups, while being essentially identical themselves, both differ substantially from the controls. This indicates that there is indeed a distinct abnormality in the serum lipids of patients with atherosclerosis and that this abnormality occurs in a relationship that is otherwise constant over all ages, numerous other diseases and several other animal species.

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TABLE I. Numbers of Subjects Studied by Age and Clinical Group.

Clinical group		Age in years: to 44	45-59	60 +	Total
No evident atherosclerosis					
Well controls	WC	38	33	8	79
Sick "	SC	21	22	37	80
WC + SC	WSC	59	55	45	159
Clinical atherosclerosis					
Myocard. infarction	MI	28	81	114	223
Cerebral thrombosis	CT	5	36	109	150
MI + CT	MCA	33	117	223	373
Total	—	92	172	268	532

Material and methods. Cholesterol was determined by the method of Pearson(6) using alcohol-acetone extracts of the serum, and phospholipids by the procedure of Lowry (7). The same laboratory methods were used throughout the study.

Clinical material was drawn from Los Angeles County Hospital and Cedars of Lebanon Hospital. Normal volunteers were found among employees of these hospitals, individuals having annual medical check-ups, and inmates and guards of the Los Angeles County Sheriff's Honor Rancho. All subjects were systematically and extensively examined by physicians regularly employed in this project.

The numbers of subjects are shown by age and clinical group in Table I. Two control groups showed no evidence of coronary or cerebrovascular disease: 79 men in apparent good health are termed "well controls" (WC) and 80 men with miscellaneous chronic diseases are termed "sick controls" (SC). The total control group is referred to as "well or sick controls" (WSC). There are also 2 groups with unequivocal clinical evidence of a major complication usually considered due to atherosclerosis: 223 men recovered from a myocardial infarction (MI) and 150 from a cerebral thrombosis (CT). The total of these 2 groups is termed "myocardial or cerebral atherosclerosis" (MCA).

Three analyses were made preliminary to those noted under Results. First, probability plots(8) were made independently of cholesterol and of phospholipids in subjects aged 45 to 59 years in each of the 4 clinical groups using the logarithms of the observed values. These showed that each variate, considered separately, tends to be normally distributed (linear) on a log scale. None of the distributions suggests serious heterogeneity. Second, a log-log scatter graph of phospholipid on cholesterol was made for the 2 control and 2 atherosclerosis groups (Fig. 1 and 2), each point representing a single case.† Fig. 1 and 2 confirm the observation of Popjak(5) that the relation between these variables seems linear when each is treated logarithmically. Third, we confirmed the finding of Popjak that the log-log linear regression of phospholipid on cholesterol appears independent of age: regressions were calculated within each clinical group for ages 44 or less, 45-59 and 60 or more and no significant age effect was found. Results are exemplified for the WC and MI groups in Fig. 3 and 4.

Regressions were calculated by the method of least squares; and t values for differences in means, regression coefficients and values of P′ calculated from regression equations for selected values of C were found in the usual way(8). A multiple t test was used for evaluation of the differences in P′ (Table IV) to make allowance for the multiple comparisons. The test used(9) takes also into account the dependence of some of the comparisons and the borderline ($P = 0.05$) heterogeneity of the variances. All computations were based on log-transformed data, the results subsequently being reconverted, where convenient, to the more conventional arithmetic units. It is obvious that a linear log-log

† Fig. 1 and 2 include also the log-log linear regressions whose constants are shown in Table II, below.

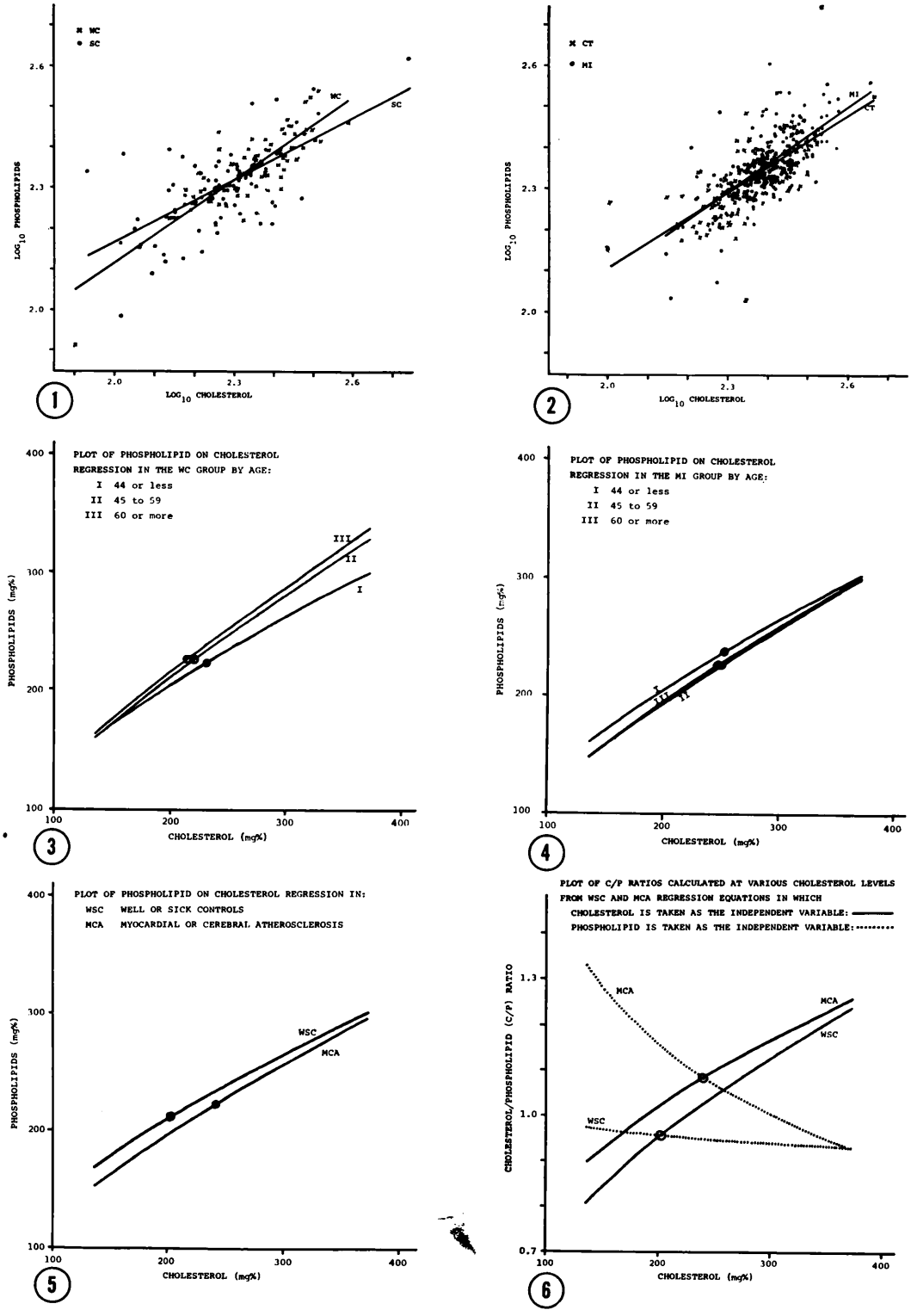

FIG. 1. Scattergraph of log cholesterol and log phospholipid levels in 2 control groups (WC and SC), and linear log-log regression in each group.

FIG. 2. Scattergraph of log cholesterol and log phospholipid levels in 2 atherosclerosis groups (CT and MI), and linear log-log regression in each group.

FIG. 3. Effect of age on phospholipid-cholesterol regression in well control group (WC).

FIG. 4. Effect of age on phospholipid-cholesterol regression in myocardial infarction group (MI).

FIG. 5. Regression of phospholipids on cholesterol in total control group (WSC) and in total atherosclerotic group (MCA). Common mean for each group denoted by a circle.

FIG. 6. Graph of C/P ratio, plotted against cholesterol, in which this ratio is found from the equation Log $P' = a + b$ Log C (*i.e.*, C independent as shown in solid line), or from the equation Log $C' = a + b$ Log P (*i.e.*, P independent as shown in dotted lines). Common means are denoted by circles.

regression will appear non-linear when presented on arithmetic ordinates.

Results. The essential statistical data required for computations are shown in Table II. The linear log-log regression of phospholipids on cholesterol is shown in Fig. 1 and 2 for each of the 4 main clinical groups. The *slopes* of these 4 curves fail to differ appreciably. The t values for differences in pairs of regression coefficients (b in Table II) are as follows: WC.SC = 1.89; WC.CT = 0.62; WC.MI = 0.26; SC.CT = 1.33; SC.MI = 2.17; and CT.MI = 0.90. The possibility that b is significantly reduced in the SC group is discounted since only one of its 3 possible differences achieves significance (and this only at the .05 level) and since the somewhat reduced b value in the SC group was found largely attributable to a single case with a low cholesterol and relatively high phospholipid (see Fig. 1).

Differences do appear to exist in the *elevation* of the curves shown in Fig. 1 and 2. The matter of differences in elevation may be examined numerically by calculating the level of phospholipids (P') in each group corresponding to any single level of cholesterol (C). This selected level should be in proximity to the cholesterol means of the 2 groups to avoid a contribution from differences in slopes of the 2 regressions. Beyond this criterion, however, there is no logical basis for preference of one particular level of C to all other levels, except that in comparing P' of 2 regressions, C might be taken at mean C for the 2 groups. In the present data cholesterol means of the separate clinical groups range from 182 to 250 mg %, while the common means of pairs of groups range from 211 to 243 mg %, the mean for the entire series of 532 cases being 229 mg %. Over this entire range, from 182 to 250 mg %, differences in observed b values make only trivial contributions to the differences in P' levels calculated from the regression equations, such differences in P' levels, therefore, representing differences in elevation (or origin with C = 0 at the selected level of C). Lacking any clear and consistent method for selecting one single level of C, we have arbitrarily chosen to make tests of differences in elevation between pairs of regressions at 0.5 standard deviations from the WC mean over a range covering the range of means of the clinical groups: namely from 1.0 σ below to 0.5 σ above the WC mean. These levels are 175, 199, 225 and 255 mg %. Significance found at any single test point is not altered in meaning by results of test at any other point: they should be viewed alternatively rather than jointly, significance at any level of C establishing significance of the difference in elevation between the two regressions.

For each clinical group, Table III shows the phospholipid level corresponding to these

TABLE II. Summary Showing for Each Clinical Group $\bar{C}$, $\bar{P}$, V_C and V_P: Means and Variances of Log Cholesterol and Log Phospholipids, Respectively; a and b: Constants of Regression Equation Log $P' = a + b$ Log C; and V_b: Variance of Regression Coefficient, b.

Group	$\bar{C}$	$\bar{P}$	V_C	V_P	a	b	V_b
WC	2.3526	2.3537	.011958	.007914	.7759	.6708	.002753
SC	2.2598	2.2984	.017041	.010682	1.1534	.5067	.004745
WSC	2.3059	2.3253	.016588	.010050	.9917	.5784	.001728
MI	2.3980	2.3591	.006780	.006750	.7060	.6894	.002359
CT	2.3578	2.3278	.009151	.007596	.8579	.6234	.002981
MCA	2.3818	2.3465	.008099	.007308	.7652	.6639	.001244

TABLE III. Levels of Phospholipids (P') Calculated from Regression Equation of Each Clinical Group for Selected Values of Cholesterol (C), C Values Being Chosen at Indicated Deviation from the WC Mean. Calculations used log values; results shown are arithmetic antilogs in mg %.

Selected cholesterol level	(C)	Corresponding phospholipid level (P') WC	SC	MI	CT	WSC	MCA
-1.0σ	175	191	195	179	181	195	180
$-.5\sigma$	199	208	208	195	195	208	195
Mean	225	226	221	213	211	225	212
$+.5\sigma$	255	246	236	232	228	242	231

selected cholesterol levels, while Table IV exhibits the significance of differences in these phospholipid levels. No significant differences occur between the WC and SC groups in phospholipid levels, nor between the CT and MI groups. Significant differences do exist, however, between the well controls and either of the 2 atherosclerosis groups, and the sick controls are almost significantly different from the atherosclerosis groups. These findings suggest formation of a combined control group, WSC, and a combined atherosclerosis group, MCA, the data for these being included in Tables III and IV. It is clear that a significant abnormality in the relationship of phospholipids to cholesterol is present in the patients with clinical evidence of atherosclerosis and that this abnormality consists of there tending to be a lower phospholipid associated with a fixed level of cholesterol. Results for the WSC and MCA groups are shown on arithmetic ordinates in Fig. 5.

Table V shows the cholesterol/phospholipid (C/P) ratios derived from the data of Table III. It is notable that in all groups the C/P ratio tends to increase with cholesterol level. At any given cholesterol level, however, the C/P ratio tends to be higher in the atherosclerotic than in the control patients. The manner in which the C/P ratio rises with increasing cholesterol is shown in the 2 continuous curves of Fig. 6 for the WSC and MCA groups.

In all of the foregoing regressions, cholesterol was taken as the independent variable —the type equation being Log P' = a + b Log C—but this was clearly an arbitrary choice. It would have been equally rational to take phospholipids as the independent variable and solve the equation Log C' = a + b Log P. These 2 alternative equations give

TABLE V. C/P Ratios (derived from data of Table III) for Clinical Groups at Selected Levels of Cholesterol (C).

C	WC	SC	MI	CT	WSC	MCA
175	.92	.90	.98	.97	.90	.97
199	.96	.96	1.02	1.02	.96	1.02
225	1.00	1.02	1.06	1.07	1.00	1.06
255	1.04	1.08	1.10	1.12	1.05	1.11

numerically quite different results, of course, because the former minimizes the regression error in P and the latter minimizes the error in C. The difference in the two approaches is magnified if one plots the C/P ratio, derived from the regression equation, against C. To demonstrate this, we have included as a dotted line in Fig. 6 the C/P ratio derived from the regression equation in which P is taken as the independent variate. This confirms the prior analysis in demonstrating a higher C/P ratio in the MCA than in WSC group. However, a strong doubt is raised of whether the C/P ratio is actually a function of the level of cholesterol. To test this further, we computed the regression Log C/P = a + b Log C. The b constants with their

TABLE IV. Differences in Phospholipid Values (P') as Shown in Table 3 for Various Selected Levels of Cholesterol (C). Significant differences are underlined, the significance level, .01 or .05 in parentheses. The statistical test used is the multiple t comparison test of Dunn(9). Critical ratios used are 3.25 for $P < .05$ and 3.70 for $P < .01$.

Level of C	WC-SC	MI-CT	WC-MI	WC-CT	SC-MI	SC-CT	WC-MCA	WSC-MCA
175	− 4	−2	+12	+10	+16	+14	+11	+15(.01)
199	0	0	+13	+13	+13	+13	+13(.05)	+13(.01)
225	+ 5	+2	+13(.05)	+15(.05)	+ 8	+10	+14(.01)	+13(.01)
255	+10	+4	+14(.05)	+18(.05)	+ 4	+ 8	+15(.01)	+11

standard deviations were as follows: WC = .2899 ± .0410; SC = .4945 ± .0689; CT = .3525 ± .0563; and MI = .3505 ± .03946. Since all these coefficients are positive and differ significantly from zero, it is concluded that the C/P ratio tends to increase with increasing cholesterol levels.

Discussion. Interpretation of these findings hinges in the first instance on the logarithmic nature of the relationships observed. For example, in each of the clinical groups the distribution of cholesterol levels among individual persons (or, separately considered, the distribution of phospholipid levels) appears normal on a log scale. The best statistic to use to indicate the "point of central tendency" is, therefore, the geometric mean rather than the arithmetic mean. More important is the fact that in a log-normal distribution deviations from the mean are *proportionally* equidistant, rather than equidistant in numbers of milligrams. Thus in the WC group, the mean is 225 while 2 standard deviations below and above the mean are 136 and 373, respectively. The former is only 89 mg below the mean while the latter is 148 mg above the mean. However, the change from 136 to 225 is proportionally exactly the same as the change from 225 to 373.

The log-normal distribution of both cholesterol and phospholipids implies that the forces that are responsible for natural variation of each variable have exponential, rather than arithmetically linear, effects upon serum lipid levels(8). In brief, cholesterol and phospholipid appear to be power functions of the biological forces determining their levels. It is not necessarily true, of course, that both kinds of lipid are regulated by the same forces. However, if each is a power function it is not at all surprising that the interlipid relationship is linear when each is treated logarithmically.

The log-log linear relation of phospholipids to cholesterol has considerable interest. It is evident that all of the regression coefficients (b values of Table II) are less than 1.0—and from the data it is readily calculated that p values for differences between these coefficients and unity are less than .001. It must, therefore, follow that if cholesterol is increased by a certain proportion over what it was, then phospholipids will also tend to increase—but by a smaller proportion. Thus the rate of increase in phospholipids is only about 2/3 the rate of increase in cholesterol. In consequence, the C/P ratio is itself dependent upon cholesterol level and can hardly have full clinical meaning by itself.

The added information provided by a regression analysis, over that yielded by the more conventional univariate methods, is exemplified by the findings in our two control groups. Here the "sick controls," presumably debilitated by chronic illnesses, have a mean cholesterol of 182 as compared with 225 in the "well controls"; a mean phospholipid of 199 compared with 226; and a C/P ratio of 0.91 compared with 1.00. These differences achieve significance at the .001 level and suggest not only that both cholesterol and phospholipids are depressed in these debilitated individuals but also, because of the lower C/P ratio, that the interlipid relationship is disturbed. The regression analysis in no way alters the fact that both lipids are depressed in the sick controls, but shows that the interlipid relationships are in no way significantly different from those found in the well controls. The lower C/P ratio is precisely what one would expect from the generally reduced lipid levels.

Our findings in the two control groups amply support the observations of Peters and Man(4) and Popjak(5) that there tends to be a fixed mathematical relationship between serum cholesterol and phospholipids. No special attention was directed in either of those studies, however, to the interlipid relations in patients with clinical manifestations of atherosclerosis. Numerous investigators, of course, have demonstrated(3) that serum cholesterol and the C/P ratio tend to be about 5 to 10% higher in patients with coronary artery disease than in control subjects. While the present data are quantitatively in agreement with these reports, they help to clarify and simplify the nature of the serum lipid abnormality found in clinical atherosclerosis.

Our data indicate that patients with myo-

cardial infarction or cerebral thrombosis are essentially identical in their phospholipid-cholesterol regressions and that both of these atherosclerotic groups differ significantly from the controls. The nature of the difference is this: that for any given level of serum cholesterol, the atherosclerotic patients tend to have a lower than normal level of phospholipids and consequently a higher C/P ratio.

In view of this abnormality in the atherosclerotic patients, it is most interesting that the relative rates of change in cholesterol and phospholipids remain undisturbed: *i.e.*, there is no significant difference in regression coefficients. Our data, therefore, suggest that patients with clinical manifestations of atherosclerosis do not differ from the controls in the responsiveness of either cholesterol or phospholipids to biological forces regulating these levels. Rather, the atherosclerotic patients tend to have a different cholesterol-phospholipid balance at every level of cholesterol. This difference in lipid balance has been observed over a broad range of cholesterol levels and may be of fundamental importance. The effect of estrogen therapy upon this interlipid relationship in men with myocardial infarction is presented separately (10).

Conclusions. 1. Serum cholesterol and phospholipid determinations were made in 4 groups of subjects: 79 normal men without evident disease ("well controls," WC), 80 men suffering from a variety of chronic disorders but without evidence of coronary artery or cerebrovascular disease ("sick controls," SC), 223 men who had recovered from a frank myocardial infarction (MI), and 150 men who had recovered from a frank cerebral thrombosis (CT). In each group the cholesterol-phospholipid relationship appeared linear on a log-log scale and linear log-log regressions were calculated for each of the 4 groups. 2. In the 2 control groups, WC and SC, showing no evidence of coronary or cerebral atherosclerosis, the quantitative relationship between cholesterol and phospholipids was the same, confirming reports of other workers. Also, age did not affect this relationship. 3. In the 2 atherosclerosis groups, a substantial and highly significant abnormality in the quantitative relationship between cholesterol and phospholipids was observed (both groups showing the same abnormality). 4. There is, therefore, a disturbance in the normal balance between these 2 kinds of serum lipid in men with atherosclerosis. This imbalance was present over the entire range of observed cholesterol levels and is thought to represent some important and consistent lipid abnormality characteristic of atherosclerosis.

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