Am J Hum Genet 28:453–464, 1976

Analysis of Genetic and Environmental Sources of Variation in Serum Cholesterol in Tecumseh, Michigan. III. Identification of Genetic Effects Using 12 Polymorphic Genetic Blood Marker Systems

CHARLES F. SING[1] AND JOHN D. ORR

INTRODUCTION

In this report we present our analysis of data collected in Tecumseh, Michigan, to determine whether any of 12 unlinked polymorphic blood and serum markers can be used to identify genetic effects on normal variation in human serum cholesterol level. This is the third report of studies designed to identify genetic and environmental factors which play a role in predicting an individual's serum cholesterol. Our findings will be compared with other studies.

POPULATION STUDIED AND METHODS OF ANALYSIS

The population of Tecumseh, Michigan, and the design for data collection have been reported previously [1–3], as have the sampling procedures, method of laboratory analysis, and distributional properties for the nonfasting serum cholesterol [4, 5]. Because of skewness in the frequency distribution of the basic data, we decided to work with the natural logarithm (ln) of the cholesterol values. Approximately 60% of the total sum of squares of ln cholesterol in either sex can be explained (in the regression sense) by variability in age, replication, and socioeconomic variation. Adjustment for these concomitant variables results in the reduction of the variance of an observation of 37% and 39% in males and females, respectively.

The increase in statistical precision for detection of correlations with genetic markers attained by adjustment of the data is obvious. However, by analyzing adjusted data we must assume that the distributions of the age, replicate, and socioeconomic variables are homogeneous among marker phenotype classes. Preliminary analyses of variance did not detect statistically significant heterogeneity of age, generation, or occupation effects among single-locus marker phenotype classes. Regardless, the comparisons among marker phenotypes presented here are conditional on the adjustments made for age, replication, and socioeconomic effects whose distributions may only reflect the community of Tecumseh and not a larger "generalized" population of inference. For this reason the comparisons with other studies, using concordance of effects to support a genetic finding, represent a conservative estimate of the role genes may play in determining normal cholesterol variation in man.

Received October 3, 1975; revised May 10, 1976.

This work was supported by U.S. Atomic Energy Commission contract AT(11-1)-1552 to the Department of Human Genetics and U.S. Public Health Service Program Project grant H-6378 to the Cardiovascular Research Center, University of Michigan, Ann Arbor, Michigan.

[1] Both authors: Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan 48104.

There were 4,619 males and 4,730 females available for adjustment by regression. Of these, 3,112 males and 3,254 females who were typed for each of the 12 unlinked polymorphic genetic markers given in table 1 were considered.

TABLE 1

ALLELES AND PHENOTYPES OF THE 12 GENETIC SYSTEMS ANALYZED

System	Alleles	Phenotypes
Gc	Gc^1, Gc^2	Gc 1-1, Gc 1-2, Gc 2-2
Gm	*A*, *B*	AA, AB, BB
Haptoglobin	Hp^1, Hp^2	Hp 1-1, Hp 1-2, Hp 2-2
MNSs	*M*, *N*	MM, MN, NN
	S, *s*	SS, Ss, ss
Rh	*C*, *c*	CC, Cc, cc
	E, *e*	EE, Ee, ee
	D, *d*	D, dd
Duffy	Fy^a, Fy^b	Fy(a+), Fy(a—)
P	P^1, P^2	P^1, P^2
ABH secretion	*Se*, *se*	Secretor, nonsecretor
Lewis secretion	*Le*, *le*	LeS, nL
Kell	*K*, *k*	K, kk
Kidd	Jk^a, Jk^b	Jk(a+), Jk(a—) Jk(b+), Jk(b—)
ABO	A_1, A_2, *B*, *O*	A_1, A_2, A_1B, A_2B, B, O

For this analysis, the Rh phenotypes were translated to the eight common phenotypes based on the R^2, R^1, R^z, and r chromosome designations. Contingency chi-square analyses to compare single-locus phenotype frequencies in this subsample with those in the total genetic sample reported by Shreffler et al. [6] failed to detect statistically significant heterogeneity ($\alpha = 0.05$ comparisonwise test criterion).

The adjusted ln cholesterol distributions for the subsample, which were also typed for the markers, had variances of 0.02908 and 0.02890 for males and females, respectively. These values do not differ greatly from 0.03054 for males and 0.02990 for females computed for the total samples. We conclude, therefore, that the ln cholesterol and marker phenotype frequency distributions for the male and female subsamples are representative of the larger samples studied by Sing et al. [4].

The strategy employed in this report for analyzing the variability in adjusted ln cholesterol values attributable to marker phenotype variability for each sex was to first consider each genetic marker system separately using the three-level nested analysis of variance of weighted means. Then the systems identified were combined to determine if there was a statistically significant improvement in prediction of cholesterol level by considering multilocus phenotypes.

Two statistical problems arise in these analyses. The first concerns the choice of an error mean square to test for significant marker phenotype variation when there is a covariance in cholesterol levels between pairs of individuals because they share a common household environment. This problem applies to the analysis of variability among both single-locus and multilocus phenotypes. The second problem concerns the difficulty (due to unequal numbers in multilocus phenotype subclasses) in estimating the deviations due to the nonadditivity of single-locus marker phenotype effects in determining multilocus marker phenotype variability in cholesterol.

To handle the first problem, the variability among household means was used to test for statistically significant differences among marker phenotype means. In this way the

covariance between individuals within a phenotype class, because they share the same household, was taken into account in the choice of the error mean square for testing marker phenotype differences. For households with more than one marker phenotype of the marker system being analyzed, one phenotype class was chosen at random to represent each household independently of the number of individuals in each phenotype class. The total sample of available cholesterol observations was reduced for each marker system by approximately 15% due to this redefinition of the sample for statistical analyses. The resulting subsamples, however, are representative of the larger sample for the genetic markers which gave statistically significant heterogeneity among phenotype means (table 2).

TABLE 2

MEAN AND STANDARD DEVIATION OF LN CHOLESTEROL FOR SAMPLES BASED ON MARKER AND HOUSEHOLD CRITERIA

SAMPLE DEFINITION	MALES			FEMALES		
	No.	Mean	SD	No.	Mean	SD
Ignoring household:						
Individuals typed for 12 markers ..	3,112	5.29757	0.17054	3,254	5.30737	0.17001
One phenotype per household:						
Individuals typed for Gm	2,605	5.29552	0.17056	2,778	5.30854	0.17140
Individuals typed for haptoglobin ..	2,543	5.29501	0.17196	2,726	5.30826	0.17128
Individuals typed for secretor	2,800	5.29721	0.16935	2,950	5.30823	0.17030
Individuals typed for ABO	2,623	5.29717	0.17027	2,784	5.30862	0.17150

The analysis of variance appropriate for detecting statistically significant variability among marker phenotypes when there is an unequal number of individuals per household and/or households per phenotype class is given in table 3. We assume that the variability

TABLE 3

ANOVA TABLE FOR TESTING MARKER PHENOTYPE EFFECTS

Source of Variation	df	Mean Squares	Expected Mean Squares
Marker phenotype	$(P\text{-}1)$	MS_P	$\sigma_{ID}^2 + q_1\sigma_H^2 + q_2\sigma_P^2$
Household within phenotype	$(H\text{-}P)$	MS_H	$\sigma_{ID}^2 + q_0\sigma_H^2$
Individuals within household	$(N\text{-}H)$	MS_{ID}	σ_{ID}^2

NOTE.—P = no. of phenotype classes; H = total no. of households; and N = total no. of individuals.

among individuals sharing the same household and among household means within marker classes are statistically independent random effects. The household mean square for testing phenotype effects was constructed as $MS_{ID} + q_1[(MS_H - MS_{ID})/q_0]$. The formula for computing q_0, q_1 and the degrees of freedom for this function are given by Sokal and Rohlf ([7], p. 274) with a general discussion of the nested analysis of variance for unequal sample sizes. Because no more than one phenotype is considered for each household,

the number of degrees of freedom available for the analysis of each single (or multiple) locus marker system is determined by the distribution among households of the marker phenotypes being analyzed. Estimates for household mean square from single-locus analyses ranged from 0.032 to 0.036. Estimates from multilocus analyses (up to four loci) fell in this range, but closer to 0.032. In every single-locus case considered, the household component of variance is statistically significant.

Rather than attempt to determine the exact proportion of sum of squares due to nonadditivity between marker systems, we chose simply to identify marker systems which gave an improved prediction when combined with other marker systems, identifying the statistically independent contribution of a marker (or combination of markers). Thus, one can infer the presence of interaction only from the failure of a marker system, which is significant by itself, to contribute significantly to reduction in variability when in combination with other markers.

The F ratio,

$$\frac{(PSS_C - PSS_R)/k}{\text{mean square household}} = F_{(k,\ \text{household df})}$$

was utilized in a stepwise fashion to identify the statistically independent contribution of markers to multilocus phenotype variability. The k coefficient is the difference in degrees of freedom for the phenotype sum of squares for the larger number of marker systems (PSS_C) and the phenotype sum of squares for the smaller number of marker systems (PSS_R). The household mean square was taken to be the estimate associated with the household sum of squares for the smaller number of marker systems. As with the single-locus marker analyses, the data represented only one multilocus marker phenotype class from each household.

RESULTS

The nested analysis of variance conducted for each genetic system identified four markers—Gm, haptoglobin, secretor, and ABO—which gave statistically significant variation among phenotypes for one or both sexes (table 4). The multilocus combinations of these systems which gave a significant reduction in residual variability beyond that identified by phenotypes based on fewer systems are also presented in table 4. The variance components σ_{ID}^2, σ_H^2, and σ_P^2 were estimated from the analysis of variance based on marker phenotypes defined by all four systems. The reduction in the variance of an individual's serum cholesterol due to fitting these marker phenotypes was 1.4% for males and 0.6% for females.

The mean and standard deviation for each phenotype for each of the four marker systems is given in table 5. These data are based on the total sample ignoring the household definition used for the statistical analyses. Standard deviations are remarkably homogeneous among phenotype classes. The phenotypes are presented in rank order determined by the sex which gave the statistically significant weighted mean square. The females determine the Gm and haptoglobin order and the males, the ABO order of presentation. The nonsecretors had a significantly greater mean cholesterol in both sexes. Scheffé's a posterori method of pairwise contrasts was used on the ranked means of the other three systems in the sex which gave statistically significant differences by the analysis of variance. The per observation error was taken to be the household mean square estimated from the nested analysis of variance. Brackets enclose those pairs of means which are not significantly different

TABLE 4

GENETIC SYSTEMS WHICH IDENTIFIED STATISTICALLY SIGNIFICANT DIFFERENCES AMONG MARKER PHENOTYPE MEANS IN ONE OR BOTH SEXES

SOURCE OF VARIABILITY	MALES				FEMALES			
	No.	df	Sum of Squares	Mean Squares	No.	df	Sum of Squares	Mean Squares
All typed individuals	3,112	3,111	90.49	0.02908	3,254	3,253	94.02	0.02890
Single-loci								
Gm	2,605	2	0.13	0.06443	2,778	2	0.22	0.11226*
Haptoglobin	2,543	2	0.12	0.06224	2,776	2	0.29	0.14261†
Secretor	2,800	1	0.38	0.37928‡	2,950	1	0.20	0.20325†
ABO	2,623	5	0.56	0.11182‡	2,784	5	0.09	0.01817
Multiloci								
Gm haptoglobin§	...	...	...	...	2,483	8	0.59	0.07396†
Gm secretor§	...	...	...	...	2,620	5	0.53	0.10687‡
Haptoglobin secretor§	...	...	...	...	2,573	5	0.40	0.08013*
ABO secretor§	2,443	11	1.18	0.10771‡	...	...	...	...
All four markers	2,150	90	3.59	0.03997*	2,321	96	3.27	0.03401

NOTE.—Data is ln cholesterol measurements adjusted for age, replicate and socioeconomic variability. Sample used for each analysis varied according to distribution of marker phenotypes among households. See text for a discussion of the analysis.

* Significance at the .05 level of probability.

† Significance at the .025 level of probability.

‡ Significance at the .01 level of probability.

§ Those combinations which gave a statistically significant reduction in residual variability beyond that removed by systems taken separately.

TABLE 5

MEAN LN CHOLESTEROL AND STANDARD DEVIATION OF PHENOTYPES FOR EACH OF THE GENETIC SYSTEMS GIVING STATISTICALLY SIGNIFICANT DIFFERENCES IN ONE OR BOTH SEXES PRESENTED IN ORDER OF RANK IN FEMALES (GM, HAPTOGLOBIN, SE) AND MALES (ABO)

PHENOTYPE	MALES			FEMALES		
	No.	Mean	SD	No.	Mean	SD
All	3,112	5.29757 (199.8)	0.17054	3,254	5.30737 (201.8)	0.17001
Gm:						
AB	1,386	5.30130 (200.6)	0.16950	1,404	5.31731 (203.8)	0.16999
AA	331	5.30394 (201.1)	0.16741	351	5.30850 (202.1)	0.16832
BB	1,395	5.29235 (198.8)	0.17232	1,499	5.29780 (199.9)	0.17000
Hp:						
22	1,050	5.30443 (201.2)	0.17628	1,129	5.32090 (204.6)	0.17127
21	1,544	5.29700 (199.7)	0.16872	1,585	5.30081 (200.5)	0.16902
11	518	5.28536 (197.4)	0.16370	540	5.29836 (200.1)	0.16716
Nonsecretor	767	5.31199 (202.8)	0.16372	831	5.32011 (204.4)	0.16766
Secretor	2,345	5.29285 (198.9)	0.17249	2,423	5.30300 (200.9)	0.17062
ABO:						
A_1	1,038	5.31445 (203.2)	0.16627	1,144	5.30878 (202.1)	0.17140
A_2	309	5.31362 (203.1)	0.16957	297	5.31517 (203.4)	0.17266
A_1B	77	5.31352 (203.1)	0.16188	83	5.29463 (199.3)	0.14812
B	301	5.30838 (202.0)	0.16579	277	5.31518 (203.4)	0.17268
A_2B	36	5.27900 (196.2)	0.15423	36	5.24830 (190.2)	0.18070
OO	1,351	5.27811 (196.0)	0.17417	1,417	5.30532 (201.4)	0.16866

NOTE.—Nos. in parentheses = anti-ln. Brackets mark those classes which are not significant using an $\alpha = 0.05$ Scheffé's test for pairs on only those genetic systems which gave statistically significant differences among phenotypes (see table 4).

when an overall $\alpha = 0.05$ experimentwise error rate is achieved for each marker system. For Gm the female phenotypes carrying the *A* allele have significantly greater mean cholesterol than the *BB* homozygote (3.58 mg/100 ml). In males this difference was concordant (1.90 mg/100 ml) but not statistically significant. For haptoglobin, in females, the 2-2 phenotype was 4.22 mg/100 ml greater than the Hp^1-carrying phenotypes. Again, this difference was not statistically significant in

males, but it was concordant (1.75 mg/100 ml). The O phenotype in males has a significantly lower mean than all but the A_2B phenotype. There appears to be no consistent rankings of ABO phenotypes between the sexes. On the average, the difference between the high and low phenotype classes for the four marker systems was 4.30 mg/100 ml for males and 6.28 mg/100 ml for females.

Every two-locus combination involving a marker system showing significant phenotype variability gave a significant improvement ($\alpha = 0.05$ level of probability) in prediction when contrasted to the reduction in sum of squares due to either of the systems in the combination. On the other hand, no three-locus combination (in females) gave a significant improvement over any of the three two-locus combinations of the three systems involved in each case. However, the four-locus combination of the four systems involved accounted for a significant reduction in variability over all two-locus combinations in males but not females.

Table 6 presents the high and low two-locus phenotypes selected on the basis of ranking in the sex which gave statistically significant two-locus marker phenotype variability (see table 4). The high and low phenotypes were concordant between sexes in every case. The average difference between the high and low phenotype classes was 8.78 and 8.90 mg/100 ml for males and females, respectively.

DISCUSSION

The proportion of the variance in the adjusted ln cholesterol value of an individual which may be assigned to variability in phenotypes based on four polymorphic genetic markers is remarkably large (approximately 1%) considering the small proportion of the human genome sampled. The evaluation of significant differences among the phenotypes of each of these four unlinked markers suggests that they identify at least as many genetic loci which influence serum cholesterol levels.

Considering the males and females as independent samples, we would expect spurious associations of cholesterol level with marker phenotypes to be randomly distributed between the samples if there were truly no marker effects. The differences among Gm, haptoglobin, and secretor phenotypes are concordant between the sexes. Only for the ABO marker system is the association heterogeneous between the sexes. However, as we shall discuss below, by considering specific contrasts among ABO phenotypes the results of this study are not inconsistent with findings from other studies. To summarize the findings of this study, it is highly unlikely, in light of the observed consistency between sexes, that the statistical differences identified by these four loci represent an expected type I error level.

A number of studies based on large samples allow a comparison among human populations for associations of serum cholesterol with marker phenotypes; the most common marker system which has been studied is, of course, ABO. In every study the *A*-carrying phenotype has had a higher mean cholesterol than non-*A* phenotypes (see [8, 9] for a review). An hypothesis, based on associations between intestinal alkaline phosphatase levels and ABO and secretor phenotypes [10–12], predicts the higher serum cholesterol levels found in Tecumseh for the A and non-secretor phenotypes. A summary of the studies compared to our data (table 7) sug-

TABLE 6

ANTI-LN VALUES FOR MEANS AND THE LOWER AND UPPER BOUNDS OF THEIR 95% CONFIDENCE INTERVALS FOR THE EXTREME TWO-LOCUS PHENOTYPES WHICH GAVE STATISTICALLY SIGNIFICANT IMPROVEMENT IN REDUCTION OF RESIDUAL VARIABILITY FOR AT LEAST ONE SEX

COMBINATION AND PHENOTYPE	MALES					FEMALES				
	No.	Relative Frequency	Mean	Lower Bound	Upper Bound	No.	Relative Frequency	Mean	Lower Bound	Upper Bound
Gm-haptoglobin:										
AB-2-2 (high)	454	0.15	203.2	200.0	206.4	470	0.14	207.9	204.9	210.9
BB-1-1 (low)	246	0.08	195.3	191.2	199.5	264	0.08	198.0	194.2	201.9
Gm-secretor:										
AA-sese (high)	76	0.02	204.6	196.9	212.6	78	0.02	210.4	203.0	218.0
BB-Se (low)	1,045	0.34	198.1	196.0	200.2	1,111	0.34	199.1	197.2	201.0
Haptoglobin-secretor:										
2-2-sese (high)	245	0.08	201.8	197.5	206.2	291	0.09	208.1	204.3	212.0
1-1-Se (low)	390	0.13	196.8	193.5	200.2	391	0.12	199.8	196.6	203.0
ABO-secretor:										
A_2-sese (high)	84	0.03	209.9	202.4	217.7	73	0.02	206.0	198.6	213.7
OO-Se (low)	1,025	0.33	194.2	192.0	196.2	1,051	0.32	199.9	198.0	201.8

NOTE.—See table 4 for the ANOVA results.

TABLE 7

ABO AND SECRETOR MARKERS AND SERUM CHOLESTEROL IN FIVE MAJOR STUDIES COMPARED TO TECUMSEH

STUDY	SEX	A AND AB		B AND O		NONSECRETORS		SECRETORS	
		No.	Mean	No.	Mean	No.	Mean	No.	Mean
Tecumseh	M	1,460	203.0	1,652	197.1	767	202.8	2,345	198.9
	F	1,560	201.92	1,694	201.73	831	205.6	2,423	200.9
[13]	M	2,317	212.1	3,683	205.7	...	...	...	...
[14]	M	246	246.9	269	237.6	...	...	...	...
	F	157	258.0	187	248.9	64	261.0	277	250.7
[15]	M & F	199	2.96*	333	—5.17*	...	...	...	...
[16]	M	4,607	210.5	5,173	208.2	...	...	...	...
[17]	M	176	188.5	222	181.6	...	...	...	...
	F	157	198.0	187	195.5	...	...	...	...
[18]	M	148	3.81	137	3.71	38	3.88	247	3.74

NOTE.—All studies are mg/100 ml except [18] which is μmoles/ml.

* Average deviation from the linear regression of cholesterol on age.

gests consistently higher cholesterol levels in the A and nonsecretor phenotype classes.

The lower cholesterol mean for the haptoglobin 1-1 phenotype found in Tecumseh is consistent in the two previous studies that used haptoglobin as a marker [15, 17]. To our knowledge, no other study has reported a Gm effect on cholesterol variability.

A number of studies using Rh as a marker have suggested a possible Rh effect on cholesterol prediction. Comparisons are difficult because of the heterogeneity among studies for the number of antigens used. Of three studies which involved only Rh+ and Rh— typings, two were consistent with the greater mean cholesterol levels of Rh negative individuals in Tecumseh, while one was not. In all cases, including Tecumseh, the difference was small (less than 2.0 mg/100 ml) and not statistically significant. Using the Rh *C* and *c* antigens, Alfred et al. [19] reported that in three widely separated populations of British Columbia Indians, serum cholesterol was always lowest for those (males and females pooled) with the CC phenotype. For pooled sexes, CC (198.40) was also lower than Cc (201.22) and cc (201.56) in Tecumseh, although the differences are not statistically significant.

This study and other reports combine to indicate that there is now evidence that there are a number of polymorphic genetic loci randomly distributed throughout the genome which each have a small effect on serum cholesterol levels. The consistent effects over a large number of studies for ABO and a lesser number for secretor and haptoglobin suggests that they may directly influence serum cholesterol levels. The alternative hypothesis that the observed effects are due to a locus (or loci) closely linked to the marker locus becomes less plausible as the number of confirmations of specific marker phenotype effects increases. The linkage disequilibrium required to keep the locus affecting cholesterol from randomizing its alleles with respect to the marker alleles across populations would need to be like that found for the MNSs and Rh complexes.

The statistical analyses of multilocus phenotypes suggest nonindependence of the information on cholesterol among the four unlinked marker systems which gave statistically significant differences. An approximate measure of the additivity between marker effects defined by the single-locus analyses is summarized in table 8. There is a postitive linear regression of cholesterol level on the number of positive single-locus deviations represented in the four-locus phenotypes, and it is concordant between sexes. Estimation of average effects of alleles and deviations from additivity of allele effects (and/or nonallele effects) in determining multilocus phenotypes is not appropriate for this model. However, the conclusion that each of the loci has a small but additive effect on cholesterol level is documented by the consistent rank order of mean values from lowest to highest in both males and females. Correlated frequencies and/or interaction among the genes being marked could account for the deviations from regression. Although exact statements of probability on the significance of a regression constructed in this manner are inappropriate, the regression outcome is consistent with the hypothesis that polygenes are involved in determining a portion of the cholesterol variation in the Tecumseh population. The concordance between sexes for the high and low two-locus phenotypes of combinations of

TABLE 8

REGRESSION OF LN CHOLESTEROL LEVEL ON NUMBER OF POSITIVE MARKER EFFECTS DEFINED BY SINGLE LOCUS ANALYSES

No. of Positive Marker Effects	Males		Females	
	No.	Mean	No.	Mean
0	341	5.26376 (193.2)	383	5.28966 (198.3)
1	1,105	5.28543 (197.4)	1,111	5.29411 (199.2)
2	1,169	5.31072 (202.5)	1,198	5.31646 (203.7)
3	437	5.31212 (202.8)	480	5.32535 (205.5)
4	60	5.35040 (210.7)	82	5.33194 (206.8)
Linear regression coefficient	...	0.01848 (3.72)	...	0.01373 (2.79)

NOTE.—Approximate anti-ln equivalent in parentheses. Phenotypes with positive marker effects: Gm: AA, AB; haptoglobin: 22; secretor: nonsecretor; ABO: A_1, A_2, A_1B, A_2B.

markers which gave statistically significant results further indicates that the results obtained in this study are not spurious type I errors.

The associations between intestinal alkaline phosphatase, cholesterol levels in the serum, and cell surface antigens which have now been reported suggest a need for in depth biochemical studies of the role red cell antigenic factors may play in determining the levels of cholesterol in the serum. Regardless of the outcome of biochemical studies, the controversy over the genetic model which explains normal cholesterol variation appears to be weighted toward the multifactorial hypothesis: small individual differences are due to environmental and genetic factors which cumulatively effect the serum cholesterol level. In the meantime, the statistical tendency to higher serum cholesterol levels for phenotypes based on certain polymorphic markers provides a basis for predicting cholesterol for a large proportion of the population as early in life as blood typing can be carried out.

SUMMARY

Four of 12 unlinked polymorphic marker systems were identified as predictors of normal serum cholesterol levels. Consistent effects between males and females and with other studies suggest that these marker loci are themselves involved in cholesterol determination or are closely linked to the involved loci. Two-locus combinations suggest that an 8–9 mg/100 ml difference in nonfasting serum cholesterol may be predicted between phenotypic classes which are not rare in frequency.

ACKNOWLEDGMENT

The senior author wishes to thank Dr. E. B. Robson for her critical discussions of cholesterol research during a sabbatical leave at the Galton Laboratory.

REFERENCES

1. NAPIER JA: Field methods and response rates in the Tecumseh community health study. *Am J Pub Health* 52:208–216, 1962
2. EPSTEIN FH, OSTRANDER LO, JOHNSON BC, PAYNE MW, HAYNER NS, KELLER JB, FRANCES T JR: Epidemiological studies of cardiovascular disease in a total community, Tecumseh, Michigan. *Ann Intern Med* 62:1170–1187, 1965
3. NAPIER JA, JOHNSON BC, EPSTEIN FH: The Tecumseh community health study, in *Case Book of Community Study,* edited by KESSLER II, LEVIN ML, Baltimore, Johns Hopkins Univ. Press, 1971, pp 25–46
4. SING CF, CHAMBERLAIN MA, BLOCK WD, FEILER S: Analysis of genetic and environmental sources of variation in serum cholesterol in Tecumseh, Michigan. I. Analysis of the frequency distribution for evidence of a genetic polymorphism. *Am J Hum Genet* 27:333–347, 1975
5. ORR JD, SING CF, BLOCK WD: Analysis of genetic and environmental sources of variation in serum cholesterol in Tecumseh, Michigan. II. The role of education, occupation, and income. *Soc Biol* 22:34–43, 1975
6. SHREFFLER DC, SING CF, NEEL JV, GERSHOWITZ H, NAPIER JA: Studies on genetic selection in a completely ascertained Caucasion population. I. Frequencies, age and sex effects, and phenotype associations for 12 blood group systems. *Am J Hum Genet* 23:150–163, 1971
7. SOKAL RR, ROHLF FJ: *Biometry.* San Francisco, Freeman, 1960, p 776
8. MORTON NE: Genetic markers in atherosclerosis: a review. *J Med Genet* 13:81–95, 1976
9. MAYO O, WIESENFIELD SL, STAMATOYANNOPOULOS G, FRASER GR: Genetical influences on serum cholesterol level. *Lancet* 2:554–555, 1971
10. BAMFORD KF, HARRIS H, LUFFMAN JE, ROBSON EB, CLEGHORN TE: Serum-alkaline-phosphatase and the ABO blood-groups. *Lancet* 1:530–531, 1965
11. LANGMAN MJS, LEUTHOLD E, ROBSON EB, HARRIS J, LUFFMAN JE, HARRIS H: Influence of diet on the "intestinal" component of serum alkaline phosphatase in people of different ABO blood groups and secretor status. *Nature* 212:41–43, 1966
12. LEADING ARTICLE: Blood-groups and serum-cholesterol. *Lancet* 2:625, 1969
13. OLIVER MF, GEIZEROVA H, CUMMING RA, HEADY JA: Serum cholesterol and ABO and Rhesus blood-groups. *Lancet* 2:605–607, 1969
14. LANGMAN MJS, ELWOOD PC, FOOTE J, PYRIE DR: ABO and Lewis blood-groups and serum-cholesterol. *Lancet* 2:607–609, 1969
15. MAYO O, FRASER GR, STAMATOYANNOPOULOS G: Genetic influences on serum cholesterol in two Greek villages. *Hum Hered* 19:86–99, 1969
16. MEDALIE JH, LEVENE C, PAPIER C, GOLDBOURT V, DREYFUSS F, ORON D, NEUFELD H, RISS E: Blood groups and serum cholesterol among 10,000 adult males. *Atherosclerosis* 14:219–229, 1971
17. FRASER GR, VOLKERS WS, BERNINI LF, DE GREVE WB, VAN LOGHEM E, MEERA KHAN P, NIJENHIUS LE, VELTKAMP JJ, VOGEL GP, WENT LN: A search for associations between genetic polymorphic systems and physical, biochemical and haematological variables. *Hum Hered* 24:424–434, 1974
18. BEEKMAN L, OLIVECRONA T: Serum-cholesterol and ABO and Lewis blood groups. *Lancet* 1:1000, 1970
19. ALFRED BM, LEE M, DESAI IO: A relationship between plasma cholesterol level, latitude, and the Rhesus blood group system. *Hum Biol* 46:641–646, 1974