Familial Hypercholesterolemia (One Form of Familial Type II Hyperlipoproteinemia)

A STUDY OF ITS BIOCHEMICAL, GENETIC, AND CLINICAL PRESENTATION IN CHILDHOOD

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ABSTRACT Primary hyperbetalipoproteinemia (type II hyperlipoproteinemia) is a common disorder associated with premature vascular disease. It is frequently due to genetic abnormalities, some of which are expressed in childhood. We have examined the manner in which that form of hyperbetalipoproteinemia known as familial hypercholesterolemia may be expressed in 236 children aged 1–19 born of 90 matings in which one parent had hyperbetalipoproteinemia of this variety and one parent did not.

Two Gaussian populations were fitted to the distribution of both low density lipoprotein cholesterol (C_{LDL}) and plasma cholesterol (C) in these children and a likelihood ratio test strongly favored a two over a one population model for both C_{LDL} ($X^2 = 18.41$, $P < 0.0005$) and C ($X^2 = 7.81$, $P < 0.025$). 45% of the children were in the population identified as affected; their mean C_{LDL} was 229. The remaining 55% were in the normal population with a mean C_{LDL} of 110 which was indistinguishable from that of an unrelated control population, aged 1–19. On the basis of an assumed frequency of hyperbetalipoproteinemia in the general population of 5%, the Edwards' test indicated that a polygenic model of inheritance was highly unlikely (expected, 22%; observed, 45%).

The segregation ratio obtained from the derived intersection between the two population curves (C_{LDL}, 164 mg/100 ml; C, 235 mg/100 ml) was 45/55 (abnormal/normal). The percentage of abnormal children in the first decade (52%) significantly exceeded that in the second (39%) ($P < 0.01$). The ratios (II/N) were 50/47 and 55/84 in the offspring of affected female and male parents, respectively ($X^2 = 3.819$, $0.05 < P < 0.10$). Only 10% of hyperbetalipoproteinemic children were considered to have hyperglyceridemia. These children, frequently, but not invariably, had a parent with hyperglyceridemia in addition to hyperbetalipoproteinemia ($P < 0.05$). None of the affected children who were examined had ischemic heart disease (IHD) and 7% had tendon xanthomas. Half of the parents (mean age, 37.4 yr) who were examined had IHD and three-quarters had xanthomas.

The data agree well with the hypothesis that hyperbetalipoproteinemia is inherited as a monogenic trait with early expression in these children. More than one genetic defect within the group is not excluded, but retrospective analyses of the 345 first-degree adult relatives of the affected parents indicated that most of the abnormal parents probably represented familial hypercholesterolemia, rather than combined hyperlipidemia, the other most generally recognized form of familial hyperbetalipoproteinemia.

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INTRODUCTION

Primary hyperbetalipoproteinemia (type II hyperlipoproteinemia) is a common disorder (1). It is frequently due to genetic abnormalities and, as such, may be ex-

TABLE I

Biochemical and Clinical Characteristics of the Affected Parents of the Children

Sex	Number	Mean age	Percentile distribution of low density lipoprotein cholesterol levels*				Clinical data‡		
			>99.9	>99	>97.5	>95	Xanthomas	Arcus	Ischemic heart disease§
Male	47	39.2	39	7	0	1	33	26	27
Female	41	35.1	38	2	0	1	22	15	9
Total	88	37.2	77	9	0	2	55	41	36

* The percentiles are age and sex adjusted and derived from a normal unrelated control population previously published (2).

‡ Of the 88 parents, sixteen (2 male, 14 female) were not examined by us. The mean age of the 72 who were examined was 37.4 yr (38.8 and 35.1 yr for males and females, respectively).

§ Ischemic heart disease is defined as documented myocardial infarction or the presence of angina pectoris. Mean ages of the parents in this group were 39.7 and 41.3 years for males and females, respectively.

pressed very early in childhood. An unknown number of genetic defects underlies familial hyperbetalipoproteinemia. One form is most generally known as familial hypercholesterolemia, essential hypercholesterolemia, or hypercholesterolemic xanthomatosis. It is attended by relatively severe hyperbetalipoproteinemia which appears consistent with a single abnormal autosomal gene (2–5), although arguments have been advanced for polygenic determination of this disorder (6). Familial hypercholesterolemia is associated with increased hazard for premature ischemic heart disease (7). The lipoprotein excess can often be reduced by appropriate therapy that has also been shown to be effective in children (8–10). Hyperbetalipoproteinemia has also been recently reported to be one phenotypic expression of another apparent single gene defect, the "combined hyperlipidemia" of Goldstein, Schrott, Hazzard, Bierman, and Motulsky (11). The expression of this disorder is usually delayed beyond childhood.

We have undertaken in the present study to examine the manner in which that form of hyperbetalipoproteinemia known as familial hypercholesterolemia may be expressed in a large group of children who had one parent with hyperbetalipoproteinemia and one with normal concentrations of low density (beta) lipoproteins. The pool of affected parents was selected without reference to involvement of their children. The latter included 236 subjects from 1 through 19 yr of age.

Plasma concentrations of cholesterol, triglyceride, and three lipoprotein classes were determined. Observed distributions were tested for fit with monogenic and polygenic models of inheritance. The presence or absence of hyperbetalipoproteinemia in a child was established with a cut-point derived from the distribution of low density lipoprotein concentrations. Lipid and lipoprotein measurements were compared as indicators of abnormality. The biochemical data were supplemented with clinical information in many of the children. This represents the only systematic study of lipoproteins in a large group of children at genetic risk for hyperbetalipoproteinemia.

The data are consistent with the view that there is early expression of hyperbetalipoproteinemia inherited as a Mendelian trait in these children. Retrospective analyses of the 345 first-degree adult relatives of the affected parents indicated that most of the abnormal parents represented familial hypercholesterolemia rather than combined hyperlipidemia. More than one genetic defect within the group is not excluded, however, since prediction of familial involvement suffers from the absence of any biochemical tests which differentiate specific forms of familial hyperbetalipoproteinemia.

METHODS

Patient population. The subjects forming the population of this study were derived from kindreds in a registry of patients with primary hyperbetalipoproteinemia, i.e, high beta lipoprotein levels not secondary to other known diseases, who had been referred to the Molecular Disease Branch of the National Heart and Lung Institute. Registration required only the presence of primary hyperbetalipoproteinemia and the affected parents in this study were selected on this basis. Nearly all were Caucasians, the majority from metropolitan Washington, D. C., Maryland, Virginia, or West Virginia.

The adult propositi had either type IIa hyperlipoproteinemia (hyperbetalipoproteinemia with normal plasma triglyceride concentrations) or type IIb (hyperbetalipoproteinemia with hyperglyceridemia) (12). The criteria for abnormality were arbitrarily chosen as the 95% age-adjusted upper limits of plasma concentration of low density lipoprotein cholesterol (C_{LDL}) [1] and triglycerides (TG) (2). All but two of the parents with type II hyperlipoproteinemia had a C_{LDL} above the 99th percentile and most were

[1] *Abbreviations used in this paper:* C, plasma cholesterol; C_{HDL}, high density lipoprotein cholesterol; C_{LDL}, low density lipoprotein cholesterol; C_{VLDL}, very low density lipoprotein cholesterol, all in mg/100 ml; p, population frequency; TG, triglycerides.

above the 99.9th percentile (Table I). The spouses of these propositi included some subjects with endogenous hyperglyceridemia and normal low density lipoprotein concentrations (type IV hyperlipoproteinemia) (12). There were no individuals with hyperlipoproteinemia of types I, III, or V (2).

The two most generally recognized syndromes or models of familial hyperbetalipoproteinemia are familial hypercholesterolemia (essential hypercholesterolemia, hypercholesterolemic xanthomatosis) and what has been recently defined as combined hyperlipidemia (11). In the latter syndrome, about one-third of affected subjects have type IIa, type IIb, and type IV hyperlipoproteinemia, respectively, and tendon xanthomas are uncommon (11). In an attempt to relate the parent population under study to these two syndromes, we retrospectively examined data from 345 first-degree adult relatives of the affected parents. In 245 the lipoprotein patterns were known. Of these, there were 96 with the IIa pattern, 22 with IIb, and 16 with IV, and 101 whose patterns were normal.[2] 55 of the affected parents in this study had tendon or subcutaneous xanthomas, 17 were free of xanthomas, and 16 were not examined by us (Table I). Of the latter 33 parents in whom xanthomas were absent or not documented, 16 had at least one adult first-degree relative with xanthomatosis and hyperbetalipoproteinemia. Therefore, 71 of the 88 parents had xanthomas or an adult first-degree relative with xanthomas. Of the affected parents who were examined by us, 60% of the male and 33% of female parents had signs and symptoms of ischemic heart disease (Table I). We concluded that most of the abnormal parents represented familial hypercholesterolemia. This presumption is based on the high frequency of xanthomas and hyperbetalipoproteinemia in the kindreds and the relatively low frequency of hyperglyceridemia. This does not imply that the parents represented a single genetic disorder. Indeed, within the models of familial hypercholesterolemia and combined hyperlipidemia, genetic heterogeneity may become evident as the specific mechanisms of familial hyperbetalipoproteinemia are elucidated.

In the registry as of January 1972 there were 240 children who met the following criteria of selection: (*a*) he or she was between 1 and 19 years of age; (*b*) *both* parents had their lipoprotein patterns determined in our laboratory; (*c*) one parent had primary type II hyperlipoproteinemia; and (*d*) the other parent had a normal C_{LDL}. Two of these children were propositi and were therefore excluded from all analyses. All but two of the children who otherwise qualified were studied (ascertainment > 99%), and these 236 subjects form the basis for this report.

Sampling. Blood samples were taken from all subjects after an overnight fast of at least 12 h. They had been instructed to eat a usual American diet for at least 1 wk beforehand. None of the subjects was receiving any lipid lowering medication. Venous blood (30–35 ml) was collected in tubes containing 0.1 ml of 15% EDTA. Plasma was separated from the red cells after centrifugation at 4°C. Of the samples obtained from children, 70% were obtained locally as were 74% of those obtained from parents. Plasma from others was shipped by air mail special delivery to Bethesda on wet ice and kept at 4°C until analyzed.

[2] Stone, N., R. I. Levy, D. S. Fredrickson, and J. Verter. Unpublished data.

The index or initial sample of each subject in the study was selected for data analysis. An exception was made to this in a few subjects in whom the second sample was used because the full complement of lipoprotein measurements had not been determined in the first sample. The second sample was only selected provided that the patient was still on a regular diet and there was no significant change in the plasma lipids.

Plasma lipid and lipoprotein determinations.[3] 96% of the subjects were studied between August 1965 and December 1971. During this time two standard plasma pools were always included in each analytical run. The acceptible coefficient of variation (c = standard deviation/mean) for both cholesterol and triglyceride was 0.03 and deviation of more than 3% from the cholesterol or triglyceride target values required that the run be repeated. The stability of mailed plasma samples was periodically checked in plasma samples which were sent to the Donner Laboratory in Berkeley, Calif and returned to Bethesda for reanalysis. There was no systematic change in the concentrations of plasma lipids or lipoproteins or in the refractive index or density of the solution with mailing.

Cholesterol was determined in a Technicon AutoAnalyzer I (Technicon Instruments Corp., Tarrytown, N. Y.) by the ferric chloride technique (13). Glycerides were measured by a fluorometric method (14). The C_{HDL} was determined on the supernate after heparin manganese precipitation of the other plasma lipoproteins (15). 5 ml of plasma, without a change in density, was subjected to preparative ultracentrifugation for 16 h at 100,000 *g*. The C_{LDL} and very low density lipoprotein cholesterol (C_{VLDL}) were then determined as outlined previously (2). Paper electrophoresis was performed in Beckman Durrum cells (Beckman Instruments, Inc., Fullerton, Calif.) using albuminated buffer (16). Of the 236 children, 19 did not have a C_{LDL} determined in the ultracentrifuge. In 3 of these 19, a high density lipoprotein cholesterol (C_{HDL}) value was available and C_{LDL} was estimated according to the formula: $C_{LDL} = C - (TG/5 + C_{HDL})$, where C represents the plasma cholesterol concentration (17). In the remaining 16, the concentration of the plasma cholesterol was used as the criterion to judge the presence or absence of hyperbetalipoproteinemia.

Test for a mixture of two distributions. The C and C_{LDL} data from the children were pooled and analyzed for the presence of a mixture of two or more populations by the maximum likelihood method of Murphy and Bolling (18). The method estimates the parameters for a mixture of two Gaussian distributions. Analyses were performed separately in which the variances were either unconstrained or constrained to be equal. Usually constraint is necessary to minimize the risk of obtaining an absurd result. Since variances within two distributions are rarely identical it is more appropriate to leave them unconstrained. There was little difference between the two analyses, however, and the results with the variances unconstrained were utilized.

Misclassification. The percentage of children "misclassified" due to the incomplete separation of apparent phenotypes (Figs. 1 and 2) was calculated from the following formula: $D = (A - B)/C$, where A is the point of minimal misclassification (i.e., the point of intersection of the two derived curves), B is the mean of the left (or

[3] The individual lipid and lipoprotein measurements from the children and their parents have been deposited with the National Auxiliary Publications Service.

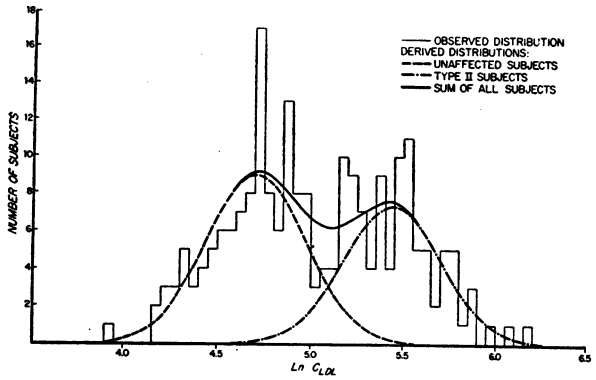

FIGURE 1 Distribution of low density lipoprotein cholesterol in the children. The natural logarithm (ln) of the low density lipoprotein cholesterol (C_{LDL}) from 217 children is plotted on the abscissa. The observed distribution appears bimodal and two populations are derived by the maximum likelihood method (17). The sum of the two derived distributions is bimodal. The antimode is a C_{LDL} of 164 mg/100 ml and 55% of the observations are in the left distribution. 7.2% of the children in the normal (left) population were above the cutpoint (false positives) and 9.7% of those in the affected (right) population were below the cutpoint (false negatives).

right) population, and C is the standard deviation of the left (or right) population. From D and a standard table (19) was derived the percentage of the left (or right) population which lay to the right (or left) of the intersect of the two curves. The sum of these percentages is the total percentage "misclassified."

Likelihood ratio test. A likelihood ratio test was used to test the fit of the C and C_{LDL} data from the children with one population and two population models. The following formulas were employed.[4]

(*a*) For the "single population" hypothesis the parameters were calculated by:

$$\hat{\mu}_0 = \frac{\Sigma x}{n},$$

and

$$\hat{\sigma}_0^2 = \frac{\Sigma x^2 - \frac{(\Sigma x^2)}{n}}{n}$$

The likelihood (L) of the data (D) is:

$$L(D/H_0) = \prod_{i=1}^{n} \frac{1}{\hat{\sigma}_0\sqrt{2\pi}} \exp\left[-\tfrac{1}{2}\left(\frac{x_i - \hat{\mu}_0}{\hat{\sigma}_0}\right)^2\right]$$

(*b*) For the two population hypothesis, the parameters were estimated by the maximum likelihood method (18). The variance was constrained to be equal since this provides a more stringent test of the two population hypothesis. The maximum likelihood was given by:

$$L(D/H_A) = \left(\frac{1}{\hat{\sigma}_1\sqrt{2\pi}}\right)^n \prod_{i=1}^{n} \left\{\hat{p} \exp\left[-\tfrac{1}{2}\left(\frac{x_i - \hat{\mu}_1}{\hat{\sigma}_1}\right)^2\right] + (1-\hat{p}) \exp\left[-\tfrac{1}{2}\left(\frac{x_i - \mu_2}{\hat{\sigma}_1}\right)^2\right]\right\}$$

(*c*) The test of the one population model hypothesis, H_0 ($\mu_1 = \mu_2 = \mu_0$) against the alternate hypothesis H_A ($\mu_1 \neq \mu_2$) is the likelihood ratio R:

$$R = \frac{L \max(D/H_0)}{L \max(D/H_A)}$$

If H_0 is true,

$$-2 \ln R \sim X_2^2$$

the degrees of freedom, 2, being the difference in the dimensionality of the parameter spaces, two parameters being estimated under H_0 compared with four under H_A.

Other tests. Means, standard deviation (SD), standard error of the means (SEM), and t tests were computed by using standard formulas (20). Smooth distribution curves were derived by calculating a series of data points with the parameters generated by the maximum likelihood method (18) and a standard formula (20). The curves connecting the data points were drawn by hand. The regression analyses of the plasma lipids and lipoproteins on age and sex were performed by using a Biomedical Computer Program (21).

RESULTS

The distributions of cholesterol and C_{LDL}. The observed distribution of the logarithms of C_{LDL} in the children appeared bimodal (Fig. 1). The parameters of two Gaussian populations represented in the sample values of C_{LDL} were estimated by the maximum likelihood method (18). Two lognormal distributions were derived (18) and the sum of these two distributions was bimodal (Fig. 1). The geometric means, that is, the antilogarithms of the means of the logarithms (4.70, 5.44) of the left and right population, respectively, were 110.3 and 229.3.[5] 55% of the children were found in the left (unaffected) population, a proportion not significantly different from the 50% predicted by a monogenic trait which segregates as a Mendelian character ($Z = 0.9447$, $P = 0.255$).[6] The derived curves intersected at a C_{LDL} of 164 mg/100 ml. The total percentage of children "misclassified" based on the parameters of the distribution curves (see Methods) for C_{LDL} was 16.9%, 7.2% of the children in the normal (left) population

[4] σ_0^2 and μ_0—the variance and mean of the distribution under the one population hypothesis (H_0); σ^2, μ_1, and μ_2—the variance and means of the distributions under the two population hypothesis (H_A); n is the number of observations; Σ_x is the sum of the observations; p is the proportion of the observations in the left population; $1 - p$, the proportion in the right population. The hat (or circumflex) over a parameter denotes the maximum likelihood estimate of that parameter.

[5] The antilogarithm of the mean of the log values comprising a sample is termed a geometric mean. It is lower than the arithmetic mean of the corresponding antilog values.

[6] The Z test is based on the maximum likelihood estimate of p and the estimate of its variance which is included in the information matrix of the Murphy-Bolling program (18).

were above the point of intersection (false positives) and 9.7% of those in the affected (right) population were below the intersection (false negatives).

The observed distribution of the logarithms of cholesterol did not so clearly represent more than one population. Two Gaussian populations were derived by the maximum likelihood method (18). The sum of the two curves was not bimodal (Fig. 2), but there was a single line (other than the x axis), tangent to the curve at two distinct points. Thus the curve for cholesterol may be considered "bitangential," evidence for a mixture of distributions even where bimodality is absent (22). The antilogs of the means of the logarithms (5.20, 5.66) of the left and right populations, respectively, were 181 and 288 and 59% of the children were found in the left population. The intersection of the derived curves occurred at a C of 235 mg/100 ml. The percent of children "misclassified" using the parameters of the cholesterol distribution curves was 27.4%; 8.5% of the children were false positives and 18.9% false negatives.

Likelihood ratio test. Although the distribution of C_{LDL} and, to a lesser extent, the distribution of C as observed or derived from the maximum likelihood method gave support to the presence of at least two populations, this was further examined by employing a likelihood ratio test (see Methods). For C_{LDL}, the likelihood ratio for a one population model (R) was 0.0001; for C, R was 0.0202, values that made it highly unlikely that only a single population was present. There was a much greater likelihood that two populations were represented (for C_{LDL}, $X^2 = 18.4064$, $P < 0.0005$; and for C, $X^2 = 7.808$, $P < 0.025$).

Comparison of unaffected population with unrelated control population. If the presence or absence of hyperbetalipoproteinemia in these children is due to a single gene, the frequency distribution of the unaffected (left) population (Fig. 1) should be the same as that in an unrelated control population. The distributions of C and C_{LDL} in an unrelated control population aged 1–19 previously studied (2) were compared with those in the left hand (unaffected) population in the present group of children. There was no significant difference between the geometric means of the two populations.

Segregation ratio. The preceding evidence for a major gene effect, obtained from the analyses of the pooled C_{LDL} and C data of the children, was further checked by performing a series of segregation ratio analyses (ratio of affected to nonaffected children). These provided an assessment of the effects of age, sex, kind of parental mating, birth order, and sibship size on the expression of hyperbetalipoproteinemia in the children. For the purposes of the analyses, a child was considered affected or nonaffected according to whether the C_{LDL} value fell above or below the experimentally

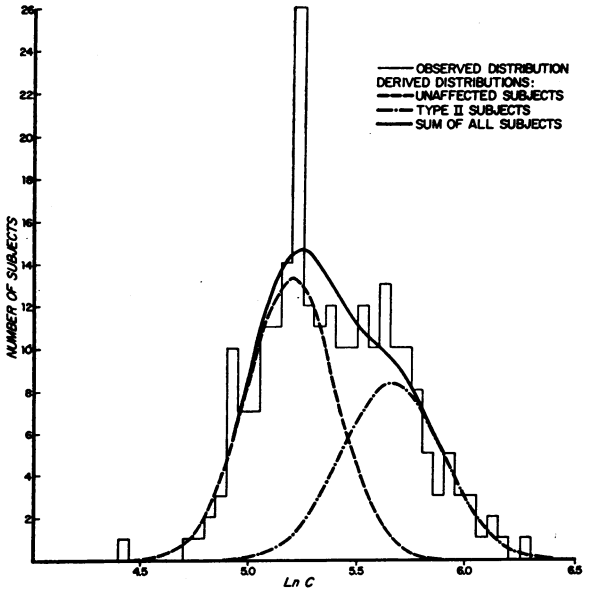

FIGURE 2 Distribution of cholesterol in the children. The natural logarithm (ln) of the plasma cholesterol (C) from 236 children is plotted on the abscissa. The observed distribution suggests bimodality and two populations are derived by a maximum likelihood method (17). The degree of overlap is sufficiently great so that the sum of the two populations is not bimodel but bitangential (see Results). The antimode for C is 235 mg/100 ml. 8.5% of the children in the normal (left) population were above the cutpoint (false positives) and 18.9% of the children in the affected (right) population were below the cutpoint (false negatives).

derived intersection between the two curves for C_{LDL} ($C_{LDL} = 164$ mg/100 ml, Fig. 1) or C ($C = 235$ mg/100 ml, Fig. 2) if a C_{LDL} was not available. The conclusions from the results that follow were not changed if one employed as a cutpoint the upper 5% of an unrelated control population ($C_{LDL} = 169$ ml/100 ml, $C = 230$ mg/100 ml) previously published (2).

The ratio of children with hyperbetalipoproteinemia to those with normal C_{LDL} was 105:131 (0.80), a value not significantly different from 1.0 ($X^2 = 2.8644$), the ratio predicted for a dominant trait. There was a significantly lower proportion ($P < 0.01$) of affected to nonaffected children in the second decade (39% affected) compared to the first (52% affected) decade (Table II). A smaller proportion of males was affected compared with females in both decades, but this difference was not statistically significant.

The segregation ratios by mating according to subtypes of hyperbetalipoproteinemia are found in Table III. The 136 children born to 55 IIa × non-type II matings were compared with the 100 from 35 IIb × non-type II matings. There was no significant difference

TABLE II

Segregation Ratio Analyses by Sex and Decade*

	Both decades		First decade		Second decade	
	Male	Female	Male	Female	Male	Female
All children	117	119	56	47	61	72
Non-type II	67	64	27	22	40	42
Normal	66	62	26	22	40	40
Type IV	1	2	1	0	0	2
Type II	50	55	29	25	21	30
Type IIa	48	47	29	23	19	24
Type IIb	2	8	0	2	2	6
Ratios	0.75	0.86	1.07	1.14	0.53	0.71
	0.80		1.10		0.62	

* In these analyses and those found in Tables III through VI, cutpoints which minimize misclassification were derived by the Murphy-Bolling program for low density lipoprotein cholesterol, C_{LDL} = 164 mg/100 ml, and cholesterol, C = 235 mg/100 ml. A child was considered to have hyperbetalipoproteinemia (type II hyperlipoproteinemia) if the $C_{LDL} > 164$; in 16 children for whom no C_{LDL} was available, a $C > 235$ was used to define abnormality. Type IIa signifies hyperbetalipoproteinemia with normal glyceride concentrations. Some of the children had a plasma triglyceride concentration above 140 mg/100 ml, a cutpoint representing the upper 5% of an unrelated control population (2); if they also had hyperbetalipoproteinemia they were classified as type IIb; otherwise they were classified as type IV hyperlipoproteinemia.

($X^2 = 0.4421$) in the segregation ratios (II/N). However, a child of a parent with type IIb was more likely to have type IIb than a child of a type IIa parent, ($X^2 = 5.5506$, $P < 0.05$). In thirteen of the 55 type IIa × non-type II matings, the non-type II parent had mild hypertriglyceridemia (type IV), but these 12 matings did not produce any type IIb children. The children were also classified according to their birth order and the ratio of affected to nonaffected children was not significantly different ($X^2 = 1.7940$) when the first born were compared to the second born.

Segregation analyses, performed according to sibship size (Table IV) gave results that were not significantly different from those expected by a hypothesis of Mendelian dominance. The possibility that a few sibships with a large number of affected children were responsible for the segregation ratio was tested by performing a segregation ratio analysis within sibships (Table V). For example, in the 32 sibships of size two, one would expect 16 sibships to have one affected and one normal child, with the remaining 16 equally divided between those with both children either affected or normal. In sibships of size two, three, and four, the observed proportions of affected: normal were not significantly different from those predicted by a monogenic trait (Table VI). We therefore concluded that the presence of approximately half of the children in the right hand (affected) population represented a general feature of the entire group and was not due to excessive contribution of affected children from a few sibships.

Effect of sex of affected parents. Segregation ratio analyses were also performed in the children according to the sex of the hyperbetalipoproteinemic parent. The ratios (II/N) were 50/47 and 55/84 in the offspring of affected female and male parents, respectively. This interesting difference is perhaps biologically significant ($X^2 = 3.319$, $0.05 < P < 0.10$).

The possibility that this difference might be related to genetic heterogeneity was investigated. For this purpose, we assumed that the occurrence of xanthomas and hyperbetalipoproteinemia together was more likely to represent a single genetic disorder than the presence of hyperbetalipoproteinemia alone. The ratios (II/N) in the children born to the 23 affected female parents with and the 5 without xanthomas were 24/24 and 9/9, respectively. The ratio was 18/14 in the offspring of the remaining 14 affected mothers who were not examined by us; however, 11 of the 14 had at least one affected first-degree relative with xanthomata. The ratio was 41/56 in the children of the 33 male parents with xanthomas and 9/26 in the children of the 12 without xanthomas ($X^2 = 2.999$, $0.05 < P < 0.10$). Only 1 of the 12 without xanthomas had an affected first-degree adult relative with xanthoma. Half of the 10 children of the two unexamined male parents had hyperbetalipoproteinemia. These data suggest but do not prove that the discrepant segregation ratios in offspring of male and female parents may be explained by the fact that the female parents appear to represent a more genetically homogeneous group.

In this regard, it is noteworthy that as a group the male parents and female parents had comparable mean C_{LDL} values (Table IX). There was a larger number of males whose C_{LDL} concentrations fell below the 99.9 percentile of the controls (Table I). The segregation ratio

TABLE III

Segregation Ratio Analyses by Kind of Parental Mating

	Children			
	Type II		Non-type II	
Parents	IIa	IIb	Normal	IV
IIa × non-type II (n = 55)	56	2	76	2
IIa × N (n = 42)	42	2	60	0
IIa × IV (n = 13)	14	0	16	2
IIb × non-type II (n = 35)	39	8	52	1
IIb × N (n = 34)	38	8	51	1
IIb × IV (n = 1)	1	0	1	0

n = number of matings. There was no significant difference in the ratio of type II to non-type II children born of different kinds of parental matings; however, a child of a parent with type IIb hyperlipoproteinemia was more likely to have type IIb than a child of a type IIa parent ($P < 0.05$).

of the offspring of these eight males was (II/N) 11/22 compared to 43/62 in the progeny of the males having C_{LDL} above the 99.9 percentile. Severity of hyperbetalipoproteinemia alone did not appear to explain an apparent deficiency of affected children of the male parents.

The possibility that the difference in the segregation ratios by sex of affected parent could explain the between decade difference was investigated. The children were divided by decade and sex of affected parent. The ratios (II/N) in children of affected female and male parents, respectively, were: age 1–9, 26/15 and 28/34; age 10–19, 24/32 and 27/50. Thus, there was a trend toward a lower ratio in the second decade for children from both parental groups which was more apparent in the affected male parental group.

Edwards' test

Threshold model. The hypothesis that the pattern of inheritance for hyperbetalipoproteinemia in these children was polygenic and only simulating Mendelism was tested by using Edwards' threshold model (23). For this purpose we assumed that the hyperbetalipoproteinemia in the parents had a population frequency (p) of 5%. Therefore, the expected number of children with hyperbetalipoproteinemia would be $\sqrt{p}$ or 22.36%. The observed values (105 children affected, 131 children nonaffected) departed significantly from those predicted by a polygenic hypothesis (52.77 affected or 0.2236×236 and 183.23 unaffected) $X^2 = 65.7949$, $P < 10^{-6}$).

Plasma lipids and lipoproteins in the children

Effect of age and sex. In these children there was no regression of any of the plasma lipid or lipoprotein fractions on age within sex. However, a regression analysis of C_{LDL} on age with the two sexes combined produced a small linear function ($C_{LDL} = 192.2–2.2$ (age)) with a decreasing slope (C_{LDL} decreased slightly with age). This regression disappeared when the normal and hyperbetalipoproteinemic children were analyzed separately (mean ages 9.2 and 10.6 yr, respectively).

The data were also analyzed for a differential influence on the C_{LDL} and C of the offspring according to the sex of the affected parent. Means for C and C_{LDL} in the children classified by sex of the affected parents were, respectively: 238.7 female parents and 225 male parents for C and 181.8 female, 161.7 male for C_{LDL}. The differences were not significant (C gave $t = 1.3465$, and C_{LDL} gave $t = 1.8473$).

Cholesterol and low density lipoproteins. Mean C and C_{LDL} were 1.70 and 2.18 times greater in the sibs with hyperbetalipoproteinemia than in their nonaffected sibs (Table VI). Mean C thus provided a less striking difference between normal and affected than did mean C_{LDL}. Children with both hyperbetalipoproteinemia and hypertriglyceridemia (type IIb) had higher mean C and C_{LDL} than did those with type IIa ($P < 0.01$ and < 0.05, respectively). When a $C > 235$ mg/100 ml was used to define abnormality the diagnoses of "affected" or "normal" differed from those obtained by C_{LDL} in 7% of the children. There were 13 children who had high density cholesterol concentrations (C_{HDL}) below the mean and in whom the C_{LDL} was underestimated from C alone. There were two children who had high C_{HDL} leading to an overestimate of C_{LDL} from C alone.

Triglycerides. The distribution of triglycerides in the children showed positive skewness, but we did not attempt to determine the presence of more than one population in the distribution. Approximately 5% of the children had a fasting $TG > 140$ mg/100 ml, a value which has been considered to represent the upper 5%

TABLE IV

Segregation Ratio Analyses by Size of Sibships

Sibship size	Ratio of affected normal		X^2	P
	Expected*	Observed		
One (18)‡	9/9	7/11	0.8889	>0.3 NS
Two (32)	32/32	31/33	0.0625	>0.8 NS
Three (19)	28.5/28.5	26/31	0.4386	>0.5 NS
Four (16)	32/32	25/39	3.0625	>0.05 NS
Five (1)	2.5/2.5	2/3	0.2000	>0.6 NS
Six (3)	9/9	9/9	0.0	>0.99 NS
Ten (1)	5/5	5/5	0.0	>0.99 NS
Totals 90	118/118	105/131	2.8644	NS

NS, not significant.

* The segregation ratio expected by the Mendelian dominant hypothesis is 1/1.

‡ Number of sibships with one child.

TABLE V

Segregation Ratio Analyses within Sibships

Sibship size	Ratio of affected/normal	Expected*	Observed	X^2	P
Two (32)‡	0/2	8	7	1.1875	>0.30
	1/1	16	19		
	2/0	8	6	(2 df)	NS
Three (19)	0/3	2	4		
	1/2	7.5	5	3.0130	>0.30
	2/1	7.5	9	(3 df)	NS
	3/0	2	1		
Four (16)	0/4	1	3		
	1/3	3	5		
	2/2	8	5	5.4167	>0.20
	3/1	3	2	(4 df)	NS
	4/0	1	1		

NS, not significant.

* Within a group of sibships of particular size, a certain proportion of the sibships will be expected by the Mendelian dominant hypothesis to represent a given ratio of affected/normal.

‡ Number of sibships of size two.

TABLE VI

Plasma Lipid and Lipoprotein Concentrations in the Children (Ages 1–19)

	Cholesterol (*C*)	Low density lipoprotein cholesterol (C_{LDL})	Triglycerides (*TG*)	Very low density lipoprotein cholesterol (C_{VLDL})	High density lipoprotein cholesterol (C_{HDL})
			mg/100 ml		
All type II*	298.7±63.0 (105)‡	241.5±60.1 (97)	81.5±50.6 (105)	14.6±10.7 (97)	43.4±12.4 (99)
Type IIa	293.9±61.9 (95)	236.9±59.2 (87)	68.5±25.4 (95)	13.2±8.9 (87)	44.5±12.5 (89)
Type IIb§	344.0±58.4 (10)	281.9±55.3 (10)	205.1±64.0 (10)	27.2±16.1 (10)	33.9±6.3 (10)
Non-type II	176.1±28.4 (131)	111.2±24.9 (120)	63.5±32.3 (130)	13.1±8.4 (120)	52.6±13.1 (121)
Normal	175.1±27.8 (128)	110.3±24.5 (117)	60.3±24.5 (127)	12.6±7.9 (117)	52.9±13.1 (118)
Type IV	218.3±20.0 (3)	145.0±14.7 (3)	198.2±38.2 (3)	32.0±7.2 (3)	41.3±3.2 (3)

* Children with hyperbetalipoproteinemia (type II) had a significantly higher mean *TG* but a lower mean C_{HDL} than their unaffected sibs ($P < 0.0025$ and <0.0005, respectively). The mean difference for *TG* disappeared if the patients with type IIb hyperlipoproteinemia were excluded from the analyses.

‡ Mean±1 SD (number of children studied).

§ Children with type IIb hyperlipoproteinemia had a significantly higher mean *C* and C_{LDL} but lower mean C_{HDL} than those with type IIa hyperlipoproteinemia ($P < 0.01$, <0.05, and <0.005, respectively).

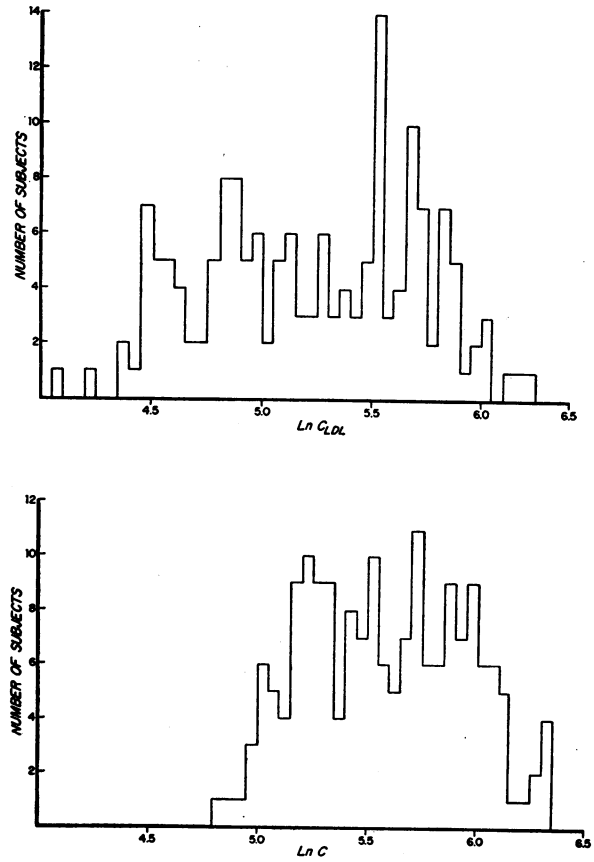

FIGURE 3 Distribution of low density lipoprotein cholesterol and cholesterol in the parents. The natural logarithm of C_{LDL} and *C* in the parents of the children is plotted. Two distributions for both C_{LDL} and *C* are expected because of the method of selection of the parents.

for triglycerides in a control population aged 1–19 (2). This was arbitrarily chosen as a cut-off point since the data in these 236 children were not sufficient to generate a more accurate internal definition of hypertriglyceridemia. Ten children were thus judged to have both hyperbetalipoproteinemia and hypertriglyceridemia (type IIb hyperlipoproteinemia). Three children with a normal C_{LDL} and hypertriglyceridemia were considered to have type IV hyperlipoproteinemia. Children with hyperbetalipoproteinemia had a higher mean *TG* than their unaffected sibs ($P < 0.0025$) (Table VI), but these differences disappeared if children with hyperbetalipoproteinemia and hypertriglyceridemia (type IIb) were excluded from the analyses. Mean *TG* of the patients with hyperbetalipoproteinemia and normal triglycerides (type IIa) and the unaffected sibs were not different.

Very low density lipoproteins. Plasma triglycerides in the fasting state are primarily carried in very low density lipoproteins. The distribution of C_{VLDL} in all the children was not obviously bimodal and there was no significant difference for mean C_{VLDL} between any of the groups of children (Table VI). Mean C_{VLDL} was, as expected, higher in the children with type IIb hyperlipoproteinemia.

High density lipoproteins. The distribution of C_{HDL} appeared unimodal. Mean C_{HDL}, however, was significantly lower in the children with hyperbetalipoproteinemia than in their unaffected sibs (Table VI) ($P < 0.005$). Children with type IIb hyperlipoproteinemia had a lower mean C_{HDL} than those with type IIa ($P < 0.005$).

Clinical data in the children

Of the 236 children, 70 of 105 considered to have hyperbetalipoproteinemia and 88 with normal C_{LDL} were

TABLE VII

Clinical Data in the Children with Hyperbetalipoproteinemia

NHLI kindred	Age	Sex	Xanthoma*	Corneal arcus	Cholesterol	Low density lipoprotein cholesterol	Triglyceride
						mg/100 ml	
2018‡	17	M	AT, ET	No	280	211	155
2021	19	F	AT, X	No	258	200	57
2021	10	F	ET, TT	No	261	211	54
2021	8	M	ET, TT	No	322	250	34
2025‡	9	M	K§	No	556	474	63
2054	15	M	AT, ET	Yes	340	282	68
2165	16	M	AT, ET, K, TT	Yes	450	392	72

* X, xanthelasma; AT, Achilles Tendon; ET, extensor tendon of hands; K, knee; E, elbow, TT, tubial tuberosity.
‡ Propositi who were excluded from the biochemical and genetic analyses.
§ Patient not examined by us but biopsy of lesion on knee determined to be a xanthoma.

examined by us. Five of the 70 effected children had xanthomas and two had corneal arcus (Table VII). Two propositi who were children and therefore excluded from the biochemical and genetic analyses also had xanthomas. The extensor tendons of the hands and the Achilles tendon were the most common site for xanthomas to occur (Table VII). None of the children had a history of angina pectoris or myocardial infarction. This was in sharp contrast to the parents, 76% of whom had xanthomas (mean age 37.4 yr) and 50% ischemic heart disease. The mean ages at which the affected males and females developed ischemic heart diseases were 39.7 and 41.3 yr, respectively (Table I). The natural history of the development of xanthomas and ischemic heart disease in the children and parents was studied by examining the prevalence of these clinical findings by decade (Table VIII).

Plasma lipids and lipoproteins in the parents

The distributions of the logarithms of C_{LDL} and C from all of the parents are found in Fig. 3. The presence of more than one mode was expected from the original selection of one abnormal and one normal parent. The right hand distribution of C_{LDL}, representing the affected parents, appeared to represent a single population, but detailed statistical analyses were not performed. The distribution of C_{HDL} contained one mode but the distributions of both C_{VLDL} and triglycerides showed considerable positive skewness.

The data on the lipid and lipoprotein fractions for all the parents are found in Table IX. The mean ages, respectively, of parents with and without hyperbetalipoproteinemia were 37.3 and 37.2 yr. In these two groups, C, TG, and C_{VLDL} were significantly higher in the parents with hyperbetalipoproteinemia (C, $P < 0.0005$; C_{VLDL}, $P < 0.0125$; and TG, $P < 0.0005$). C_{HDL} was significantly lower ($P < 0.00025$). Within the groups of parents with elevated C_{LDL}, those with hyperbetalipoproteinemia and hypertriglyceridemia (type IIb) had a higher mean cholesterol and C_{LDL} than those with hyperbetalipoproteinemia alone; however, this mean difference was not significant. Mean C_{HDL} in the type IIb parents was even lower than that found in the type IIa parents ($P < 0.05$). When the parents were further classified by sex, several interesting observations became apparent. The mean C_{LDL} in affected male and female parents were indistinguishable. Females and males with type IIb hyperlipoproteinemia had comparable mean VLDL levels; the mean C_{LDL} in the females was higher but this difference was not significant. The differences in the mean C_{HDL} between affected and normal parents seemed to reside

TABLE VIII

Comparison of Xanthomatosis and Ischemic Heart Disease in the Children and Their Parents with Hyperbetalipoproteinemia

	Xanthomas	Ischemic Heart Disease
Children*		
Age 1–9	1/38	0/38
10–19	4/32	0/32
Total	5/70	0/70
Parents‡		
Age 20–29	9/13	2/13
30–39	27/30	15/30
40–49	17/27	17/27
50–59	2/2	2/2
Total	55/72	36/72

* Of the 236 children, 70 considered to have hyperbetalipoproteinemia and 88 with normal C_{LDL} were examined by us.
‡ Of the 88 parents with hyperbetalipoproteinemia, 72 were examined by us.

TABLE IX

Plasma Lipid and Lipoprotein Concentrations in the Parents

	Cholesterol (*C*)	Low density lipoprotein cholesterol (C_{LDL})	Triglycerides (*TG*)	Very low density lipoprotein cholesterol (C_{VLDL})	High density lipoprotein cholesterol (C_{HDL})
			mg/100 ml		
All parents					
All type II*‡	367.5±77.5 (88)	297.7±77.8 (84)	148.2±75.1 (88)	26.7±17.2 (84)	43.9±12.8 (87)
Type IIa	356.7±76.6 (53)	290.9±78.7 (50)	100.3±32.9 (53)	18.5±10.5 (50)	45.9±12.8 (52)
Type IIb§	383.8±77.1 (35)	307.6±76.7 (34)	220.8±61.4 (35)	38.7±18.1 (34)	40.4±12.2 (35)
Non-type II	200.2±37.9 (90)	126.9±32.7 (82)	108.5±80.1 (89)	20.6±16.9 (82)	51.0±16.1 (82)
Normal	194.3±34.3 (76)	122.9±30.8 (69)	82.6±31.0 (75)	15.9±10.1 (69)	53.3±15.6 (69)
Type IV	232.7±41.0 (14)	148.0±36.0 (13)	247.4±115.4 (14)	45.8±23.0 (13)	39.1±13.3 (13)
Male parents					
All type II	369.3±80.5 (47)	299.0±83.4 (46)	171.4±76.7 (47)	29.0±20.0 (46)	39.9±10.3 (47)
Type IIa	362.9±92.3 (23)	299.6±95.0 (22)	112.7±28.8 (23)	17.7±12.4 (22)	42.2±10.5 (23)
Type IIb	375.3±68.8 (24)	298.5±73.2 (24)	227.6±65.0 (24)	39.4±20.3 (24)	37.7±9.8 (24)
Non-type II	210.7±41.0 (41)	140.0±33.0 (37)	140.7±103.2 (40)	27.2±20.6 (37)	40.6±9.2 (37)
Normal	200.8±35.2 (30)	134.6±30.2 (26)	93.0±28.1 (29)	18.5±10.2 (26)	42.6±8.5 (26)
Type IV	237.6±44.9 (11)	152.8±37.3 (11)	266.4±123.9 (11)	47.8±24.5 (11)	36.0±9.6 (11)
Female parents					
All type II‖	365.4±74.9 (41)	296.1±71.6 (38)	121.6±64.4 (41)	23.8±12.7 (38)	48.1±14.1 (40)
Type IIa	351.9±63.2 (30)	284.1±64.0 (28)	90.7±33.1 (30)	19.1±9.0 (28)	48.8±13.9 (29)
Type IIb	402.2±93.9 (11)	329.4±84.5 (10)	205.8±52.4 (11)	37.2±12.4 (10)	46.4±15.2 (11)
Non-type II	191.5±33.0 (49)	116.2±28.6 (45)	82.2±39.3 (49)	15.2±10.4 (45)	59.5±15.5 (45)
Normal	190.0±33.4 (46)	115.9±29.2 (43)	76.0±31.1 (46)	14.3±9.8 (43)	59.7±15.4 (43)
Type IV	214.7±14.7 (3)	121.5±2.1 (2)	178.0±25.5 (3)	34.5±0.7 (2)	56.0±22.6 (2)

* Defined as having a C_{LDL} above a boundary limit representing the upper 5% in an age matched control population previously studied (2).

‡ Parents with hyperbetalipoproteinemia had a significantly higher mean *C*, *TG*, and C_{VLDL} than the non-type II parents (*C*, $P < 0.005$; *TG*, $P < 0.0005$; C_{VLDL}, $P < 0.0125$). Mean C_{HDL}, however, was significantly lower ($P < 0.0003$).

§ Mean C_{HDL} in parents with type IIb hyperlipoproteinemia was significantly lower than in those with type IIa ($P < 0.05$).

‖ Differences in mean C_{HDL} between affected and normal parents appeared to reside primarily within the group of female parents ($P < 0.05$).

primarily within the group of female parents ($P < 0.01$); mean C_{HDL} was not different between the affected and normal males (Table IX).

Within the group of parents with normal C_{LDL}, there were 14 with hypertriglyceridemia, usually of a mild variety (mean *TG*, 247 mg/100 ml, range 144–546 mg/100 ml). These type IV parents had a higher mean *C* and C_{LDL} but lower C_{HDL} than the group of parents with entirely normal lipids and lipoproteins (*P* values, respectively, < 0.0005, < 0.01, and < 0.0025).

DISCUSSION

In this report we have examined the plasma lipid and lipoprotein concentrations in 236 children born of 90 matings in which one parent had elevated concentrations of low density lipoproteins and one parent did not. Data were presented which suggest that most of the affected parents had the form of hyperbetalipoproteinemia or type II hyperlipoproteinemia known as familial hypercholesterolemia. This assignment is not subject to specific biochemical test at the present time, however, and the degree of genetic heterogeneity in the parental pool remains unknown. If we assume that most represented familial hypercholesterolemia, it is also probable that, even as representatives of that syndrome, the abnormal parents were subject to further unintentional selection. Each parent or an adult relative had been referred to a clinic specializing in diagnosis and treatment of severe hyperlipoproteinemia, and there was a high frequency of xanthomas in the adults. Moreover, nearly 90% of the abnormal parents had a C_{LDL} concentration higher than the 99.9 percentile of an unrelated control population. In the present study, we have attempted to ascertain how cholesterol and LDL concentrations might be distributed in children with a single such affected parent, whether the distributions represented more than

one population, how the populations segregated with respect to limits generated by the data themselves appeared to relate to models of inheritance, and what correlations there were between "normals," "abnormals," and certain other variables.

The results demonstrated, for the first time, bimodality in the distribution of lipoprotein concentrations in a large group of blood relatives. In our subjects, C_{LDL} clearly provided a greater separation of the two populations than did the plasma cholesterol concentrations (C). The sum of the two curves for C_{LDL} was bimodal while the sum of the two best curves for C obtained by this method was not. However, the curve for C was touched by a tangent at two distinct points other than the x axis, a characteristic compatible with a mixture of two distributions (22). The total percentages of children "misclassified" based on the parameters of the distribution curves were 16.9 when C_{LDL} was used and 27.4 when C was employed. The apparently sharper definition of abnormality provided by LDL compared to cholesterol may be partially explained by the concomitant decrease in cholesterol associated with high density lipoprotein (HDL) in children with hyperbetalipoproteinemia, an observation previously reported in similarly affected infants (24) and adults (25).

The maximum likelihood program provided an estimate of the proportion of children in the unaffected (p) and affected (1–p) population. The overall segregation ratio of 45/55 was compatible with the presence of a major gene effect. That the ratio was less than 50/50 might be explained on the basis of either incomplete penetrance or misclassification. Misclassification of subjects is a particular problem when the phenotype is defined by using a quantitative marker. Overlap and incomplete separation of phenotypes is often the case. For example, as Murphy has previously pointed out, the distribution of galactose-phosphate uridyl transferase activity in normal subjects and carriers of the galactosemia gene is bimodal but does not separate clearly even in this well-established single locus disorder (26). In addition, there may be other loci which are modifying or preventing the expression of the mutant allele (epistasis).

Demonstration of bimodality is also not absolute evidence for a monogenic mechanism. A variety of artefacts may produce bimodality (26). Jensen and Blankenhorn believe that bimodality in families affected with familial hypercholesterolemia results from the pooling of data from families with different average serum cholesterol values (6). Alternatively, it has been pointed out, however, that intrafamily differences may tend to obscure bimodality (27). Numerous environmental and genetic factors undoubtedly affect C and C_{LDL} and the interaction of these factors in any given family is likely to be quite complex. In spite of the variety of such modulating influences, we found in this study the coexistence of children with either a significantly elevated or an obviously normal C_{LDL} in many sibships. In addition the distribution curve for C_{LDL} of the left (unaffected) population could not be distinguished from that of an unrelated control population. Furthermore, we have presented evidence that the size of the affected population was not due to excessive contribution of affected children from a few sibships (Table IV and V) and that bimodality was not related to an effect of the sex of the affected parent.

The presence of the two apparent populations among the children observed here was further tested by a likelihood ratio test. Unimodal distributions for C and C_{LDL} would be expected if these variables were determined primarily by the action of multiple loci. The one-population hypothesis was tested against the two-population hypothesis for both C_{LDL} and C. Results of the likelihood ratio test indicated that it was highly unlikely that a unimodal model could explain the observed distributions of C_{LDL} or C.

We also tested the possibility that the pattern of inheritance in these children was simulating Mendelism. The Edwards model of multiple interacting genes (23) was used, the calculations being based on the assumption that the abnormality in the affected parents occurred at a population frequency (p) of 5%. The predicted frequency of affected children was far lower than that observed, again supporting a monogenic hypothesis.

Segregation ratios for the two populations among the children were computed by two methods. One was based on the distributions generated by the maximum likelihood method (18). The other used the upper 5% from an unrelated control population as a cutpoint (2). In the first instance an individual child was considered affected or unaffected according to a C_{LDL} of 164 mg/100 ml, the nadir between the two populations. In the second analyses, a C_{LDL} of 169 mg/100 ml was used as the cutpoint. By either method the segregation ratio was not significantly different from 1.0 when the children of all ages were considered.

The evidence is convincing that in the population we have studied hyperbetalipoproteinemia (and hypercholesterolemia) is produced by mutation at a single autosomal locus, or one of several loci having similar expressivity. If we assume that familial hypercholesterolemia is the disease primarily represented here, the present study provides unequivocal confirmation of the monogenic inheritance of this disorder or group of similar disorders as suggested by previous work in other kindreds (3–6, 11).

Although the evident monogenic defect is usually sufficiently powerful to override what are undoubtedly

a multiplicity of other environmental and genetic factors controlling LDL concentrations, there may be certain unknown factors controlling expression of the mutant gene. Evidence of this was obtained by comparing the proportion of children with hyperbetalipoproteinemia at two different decades. The percent affected among children < 10-yr old (52) was significantly higher than in the children ages 10–19 (39) ($P < 0.01$). It has been previously suggested that the hyperbetalipoproteinemia in familial hypercholesterolemia is expressed quite early in life (3–5, 11, 28), and we have recently presented evidence that approximately half of the progeny of a single affected parent have elevated C_{LDL} in cord blood (24). There being no evidence of unusual mortality or morbidity in these children, the above data then suggest that influences operating during adolescence may tend to obscure hyperbetalipoproteinemia in some children. At least one longitudinal study has suggested that 80% of normal children had a decrease in plasma cholesterol during adolescence (29). The deficiency of affected subjects among older children was pursued further in connection with segregation analyses performed according to the sex of the affected parent. The latter unexpectedly revealed that the ratio of affected to normal children was considerably higher when the mother was affected than when the father had hyperbetalipoproteinemia, a difference that falls between the 0.05 and 0.10 level of statistical significance. There was a downward trend, however, in the percentage of affected children in the second decade regardless of the sex of the affected parent. We believe that suggestion of an effect of parental sex on expression may be an indication of genetic heterogeneity most marked in the male affected parents, but no proof for this could be developed.

We also examined the prevalence of concomitant hyperglyceridemia in the children and its relationship to the same phenomenon in the affected parent. It is not known what factors account for hyperglyceridemia in only some patients with familial hypercholesterolemia or combined hyperlipidemia. About 10% of the affected children also had hyperglyceridemia (type IIb) as judged by the arbitrary cut-off we employed. A hyperbetalipoproteinemic child of a parent with type IIb was more likely to have hyperglyceridemia; however, many of the children of type IIb parents had hyperbetalipoproteinemia with normal triglyceride concentrations (type IIa). A comparison of the frequency of the two patterns of hyperbetalipoproteinemia in the children and in the parents and their adult relatives suggested that the frequency of concomitant hyperglyceridemia will increase somewhat as the children grow older.

Jensen and Blankenhorn (6) have suggested that there were two groups of affected propositi (adults) in Nevin and Slack's (4) families with hypercholesterolemic xanthomatosis. In the present study, only one distribution for both C and C_{LDL} was evident in the parents with hyperbetalipoproteinemia. Noticeable skewing to the right in the distributions of both triglycerides and C_{VLDL} was mainly attributable to the significant proportion (40%) of parents with type IIb hyperlipoproteinemia. There were twice as many male parents with type IIb than females. We have no explanation for this interesting difference. Males with type IIb had a mean C_{LDL} similar to those with IIa, with the greater mean total cholesterol in IIb being entirely contributed by the C_{VLDL}. Females with IIb, however, had a higher mean C_{LDL} than those with IIa. Some of the "normal" spouses had a type IV pattern and also a higher mean C_{LDL} than those without hypertriglyceridemia. What proportion of these latter represented "sporadic" as compared to "familial" hyperlipidemia is not known; addition of the matings which included these type IV parents did not alter the segregation ratio or the phenotypic patterns of the children.

The association of familial hypercholesterolemia and premature vascular disease and the relatively high frequency of this genetic disorder (11) lend it considerable importance. In this study, we have seen a marked difference in the presence of xanthomatosis and ischemic heart disease in the children compared to their parents or other adult relatives (1). The apparent urgency of establishing prophylactic measures early creates special need for better understanding of hyperbetalipoproteinemia in children. The present study defines the frequency of abnormality, the high penetrance, early expressivity, and certain other features that occur in children of parents who appear to have a fairly uniform disorder. The data provide a base useful for comparison with information similarly gathered in children from families affected with different kinds of hyperbetalipoproteinemia, as these become better defined in both genetic and biochemical terms.

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REFERENCES

1. Fredrickson, D. S., and R. I. Levy. 1972. Familial hyperlipoproteinemia. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. Chap. 28. 545.
2. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276**: 34, 94, 148, 215, 273.

3. Khachadurian, A. K. 1964. The inheritance of essential familial hypercholesterolemia. *Am. J. Med.* **37**: 402.
4. Nevin, N. C., and J. Slack. 1968. Hyperlipidaemic xanthomatosis. II. Mode of inheritance in 55 families with essential hyperlipidemia and xanthomatosis. *J. Med. Genet.* **5**: 9.
5. Schrott, H. G., J. L. Goldstein, W. R. Hazzard, M. C. McGoodwin, and A. G. Motulsky. 1972. Familial hypercholesterolemia in a large kindred. Evidence for a monogenic mechanism. *Ann. Intern. Med.* **76**: 711.
6. Jensen, J., and A. Blankenhorn. 1972. The inheritance of familial hypercholesterolemia. *Am. J. Med.* **52**: 499.
7. Slack, J. 1969. Risks of ischaemic heart-disease in familial hyperlipoproteinemic states. *Lancet.* **2**: 1380.
8. Kwiterovich, P. O., R. I. Levy, and D. S. Fredrickson. 1970. The early detection and treatment of familial type II hyperlipoproteinemia. *Circulation.* **42**: III-11.
9. Levy, R. I., D. S. Fredrickson, N. J. Stone, D. W. Bilheimer, W. V. Brown, C. J. Glueck, A. M. Gotto, P. N. Herbert, P. O. Kwiterovich, T. Langer, J. LaRosa, S. E. Lux, A. K. Rider, R. S. Schulman, and H. R. Sloan. 1973. Cholestyramine in type II hyperlipoproteinemia. A double-blind trial of cholestyramine in type II hyperlipoproteinemia. *Ann. Intern. Med.* **79**: 51.
10. West, R. J., and J. K. Lloyd. 1973. Use of cholestyramine in treatment of children with familial hypercholesterolemia. *Arch. Dis. Child.* **48**: 370.
11. Goldstein, J. L., H. R. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52**: 1544.
12. Beaumont, J. L., L. A. Carlson, G. R. Cooper, Z. Fejfar, D. S. Fredrickson, and T. Strasser. 1970. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. W.H.O.* **43**: 891.
13. Total Cholesterol Procedures N—24b. 1964. AutoAnalyzer Manual.
14. Kessler, G., and H. Lederer. 1966. Fluorometric measurement of triglycerides. Automation in Analytical Chemistry. Technicon Symposia. 1965. L. T. Skeggs, Jr., editor. Mediad. New York. 341.
15. Burstein, M., and J. Samaille. 1960. On a rapid dosage of cholesterol read with alpha and with beta lipoproteins of serum. *Clin. Chim. Acta.* **5**: 609.
16. Lees, R. S., and T. F. Hatch. 1963. Sharper separation of lipoprotein species by paper electrophoresis in albumin-containing buffer. *J. Lab. Clin. Med.* **61**: 518.
17. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18**: 499.
18. Murphy, E. A., and D. R. Bolling. 1967. Testing of a single locus hypotheses where there is incomplete separation of the phenotypes. *Am. J. Hum. Gen.* **19**: 322. 1967.
19. Documenta Geigy. 1962. *Scientific Tables.* K. Diem, editor. Geigy Pharmaceuticals, Ardsley, N. Y. 6th edition. 28.
20. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th edition.
21. BMD Biomedical Computer Programs. 1971. W. J. Dixon, editor, University of California Publications in Automatic Computation No. 2. University of California Press, Berkeley, Calif.
22. Harris, H., and C. A. B. Smith. 1949. The sib-sib age of onset correlation among individuals suffering from a hereditary syndrome produced by more than one gene. *Ann. Eugenics.* **14**: 309.
23. Edwards, J. H. 1960. The simulation of Mendelism. *Acta Genet.* **10**: 63.
24. Kwiterovich, P. O., Jr., R. I. Levy, and D. S. Fredrickson. 1973. Neonatal diagnosis of familial type-II hyperlipoproteinemia. *Lancet.* **1**: 122.
25. Gofman, J. W., O. deLalla, G. Glazier, N. K. Freeman, F. T. Lindgren, A. V. Nichols, E. H. Strishawer, and A. R. Tamplin. 1954. The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis, and coronary heart disease. *Plasma.* **2**: 413.
26. Murphy, E. A. 1964. One cause? Many causes? The argument from the bimodal distribution. *J. Chronic Dis.* **17**: 301.
27. Murphy, E. A. 1967. Some difficulties in the investigation of genetic factors in coronary heart disease. *Can. Med. Assoc. J.* **97**: 1181.
28. Harlan, W. R., Jr., J. B. Graham, and E. H. Estes. 1966. Familial hypercholesterolemia: a genetic and metabolic study. *Medicine.* (*Baltimore*). **45**: 77.
29. Lee, V. A. 1967. Individual trends in the total serum cholesterol of children and adolescents over a ten-year period. *Am. J. Clin. Nutr.* **20**: 5.