A study of approximately 1,400 school children from 6 to 20 years of age revealed a number of interesting findings, among them differences and absence of differences in cholesterol levels. The study also suggests a need to reevaluate the concept of a causal connection between serum lipid levels and accumulation of lipids in the arterial wall.

A COMPARATIVE STUDY OF SERUM CHOLESTEROL LEVELS IN SCHOOL CHILDREN AND THEIR POSSIBLE RELATION TO ATHEROGENESIS*

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ARTERIOSCLEROTIC cardiovascular disease and its complications is the greatest single cause of mortality in the United States today. Despite the tremendous activity and research directed toward its solution, the etiology remains unknown.

One approach in attempting to elucidate its etiology is to study groups of individuals who have variations in the incidence of the disease. The mortality from cardiovascular disease, particularly coronary thrombosis, appears to be much less in the Evans County, Ga., Negro population than in its white population. This apparent racial difference has been reported by numerous observers in other areas.

A long-term epidemiological study of atherosclerosis is being conducted in this county. The areas of diet, serum cholesterol, beta lipoprotein, body build, occupation, family history, blood pressure, blood coagulation, and pathological data obtained from autopsies are now being studied or will be investigated in the near future.

Included in this profile is a comparative study of serum cholesterol and beta lipoprotein levels in the entire school population of the county. This paper will describe those observations on serum cholesterol and beta lipoproteins representing the first three grades of school and, in some instances where analysis is complete, on the entire 1,402 observations for all 12 grades of school.

Methods and Material

Evans County, Ga., has a fairly stable homogeneous population of approximately 7,500 people, two-thirds white and one-third Negro. It is a rural, nonindustrialized, high pork-consuming southern community which lends itself well to such a study. The senior author was born and has lived in the county all his life and knows intimately practically every family in the county.

This phase of the study represents 1,321 observations on serum cholesterol

* This is a preliminary report.

and 1,263 on beta lipoprotein. All blood samples (fasting) were collected at the school and immediately carried to the laboratory for centrifugation and separation of serums. Cholesterol was measured by the method of Zlatkis, Zak, and Boyle.[1]

The blood samples were in connection with a county-wide civil defense effort to type the blood of all residents. All children in school were to be included in the study. Approximately 1 per cent of the parents refused permission and these children were omitted. The child's age, weight, and height were recorded at the time the blood specimens were taken.

The alpha/beta ratios were determined by electrophoretic separation of the serum and stained according to the method of Jenks, Durrum, and Jetton.[2] The amount of dye uptake by the alpha and beta portion was determined colorimetrically after elution. The total dye uptake divided into each fraction gave the alpha/beta ratio in percentage.

Appraisal of Laboratory Methods

A. Serum Cholesterol—A study of the reproducibility of laboratory methods is essential if reliability of data is to be established. The Cooperative Study of Lipoproteins[3] highlighted the persistent difficulties with the prevailing laboratory methods for measuring cholesterol and documented the technical errors of measurement associated with each technic.

The statistic used to measure the technical error of measurement was:

$$s_e = \sqrt{\frac{\Sigma(d^2)}{2k}}$$

where d = difference between duplicates of k pairs of measurements.

The foregoing statistic assumes that the distribution of duplicate measurements is such that the average difference between pairs is zero, or negligible. The latter assumption is usually correct, but if the second determination should have a change in the average measurement because the serum has been standing longer, the above statistic would overstate the true reproducibility of the method itself. A more correct estimate of true reproducibility is:

$$s = \sqrt{\frac{\Sigma(d^2)}{2k} - \frac{k}{2}(\Sigma d)^2}$$

where d = difference beween the first and second determinations, respectively.

In the present study, both of the given technical errors of measurements were calculated to assure the investigators that d was essentially not different from zero as well as to learn the magnitude in the fluctuations of d itself.

(a) In a routine investigation of serum cholesterol reproducibility, 27 determinations were repeated on one day and the duplicates coded so that an individual's identity was unknown to the technician. The value of s_e was 3.73 and s was found to be 3.66 and the value of d was not different from zero. This compares very favorably with the technical errors of measurement encountered in the Cooperative Study where the values of s_e ranged from 5.0 to 17.3 in the four laboratories studied.

In addition, several times during the week that the laboratory performed cholesterol determinations, a small number of duplicate unknowns was included as a quality control measure to standardize procedures.

(b) To establish the validity of this laboratory's methods with that of other institutions, the Cleveland Clinic from the Cooperative Study sent three samples of serum in thermos jugs. The serum was several days old when used in the laboratory but the results were as follows:

Sample	Claxton	Cleveland Clinic
A	461	475
B	309	355
C	194	195

Also the Harvard Laboratory from the Cooperative Study ran a reconstituted lyophilized serum sample whose cholesterol was estimated by the laboratory here as 200 mg/100 ml. That laboratory made nine determinations on the sample with values ranging from 192 to 239, with an average of 197.

(c) All cholesterol measurements were made in duplicate by a single laboratory technician. If the duplicate determinations differed by more than 5 per cent, the observations were repeated the next day and averaged.

B. Beta Lipoproteins—The limitations to methods for doing lipoprotein determinations are fully appreciated. Paper electrophoresis appeared to be the most feasible for a large epidemiological study such as the Evans County study. It is recognized the dye uptake is not linear for high beta serum and different dye lots cannot be compared without standardization.

Lipoproteins, also, cannot be adequately preserved in their normal state. Therefore, they do not lend themselves easily to the running of duplicate samples over a period of days for longitudinal reproducibility studies.

However, a sample of blood was divided into 16 parts, and all determinations made at the same time. The values ranged from 84 per cent to 90 per cent with an average of 86 per cent and a standard deviation (s) = to 1.3 per cent. This means that less than 10 per cent of the time a single determination would differ from its true value by more than plus or minus 1.75 (1.3) = 2.3 per cent.

Results of Observations

Hemolysis

Of the 1,402 participating children in school in the county, blood specimens on 78 of these hemolyzed to the extent that neither cholesterol nor beta lipoproteins were measurable.. In addition, there were three instances where only the serum cholesterol could not be determined and 61 instances where only the beta ratio was not measurable.

The distribution of the 78 blood specimens by age, race, and sex is presented in Table 1.

Table 1—Distribution of Blood Specimens Which Hemolyzed, by Race, Sex, and Age Groups

Age	White		Nonwhite	
(in Years)	Male	Female	Male	Female
6	3	3	2	1
7	1	6	6	3
8	2	3	3	3
9	4	2	6	7
10	—	—	3	1
11	—*	2	2	2
12	2	3	2	1
13	—	—	—	—*
14	—	1	—	—
15	1	—	—	—
16	—	2	—	—
17	—	1	—	—
Total	13	23	24	18

* In addition to the 78 blood specimens where hemolysis had occurred as shown here, one nonwhite female aged 13 and two white males aged 11 years had blood specimens in which serum cholesterol could not be properly determined.

In comparing these 78 children in Table 1 with the remainder of the school group, there does not appear to be any association of the hemolysis with age, race, sex, blood type, height, or weight. It can be assumed for the present purpose that these missing values are randomly distributed among the county children.

Serum Cholesterol

The quickest method of describing the results is to look at the group averages by age, race, and sex. In Table 2, the average serum cholesterol values are thus presented.

It can be seen in Table 2 and Figures 1 and 2 that the serum cholesterol levels increased with age among each of the four race-sex groups. The rate of increase was slightly faster among the nonwhite children, since the rate was (3.3-4.3) units in contrast to (2.7-2.8) units per year for white children. There was a tendency for the females to increase slightly faster than males but in neither sex is the difference a significant one.

In terms of an over-all sex differential, there is no significant or consistent difference in either race. Similarly, a crude examination would suggest that there exists no racial differential. This is misleading, however, because the nonwhite race is definitely lower in cholesterol during the first two or three grades of school but by the age of nine years the difference has vanished. (Could this be the effect of a school lunch and milk program?) A smaller difference begins to appear once more around ages 13 through 16. This peculiar interaction is explained partially by the differences in rate of increase in serum cholesterol. As shown in Figure 1, the white race has a steady rate of increase. In contrast, Figure 2 shows that the nonwhite children have an accelerated spurt from six to nine years of age and then a decelerated growth rate.

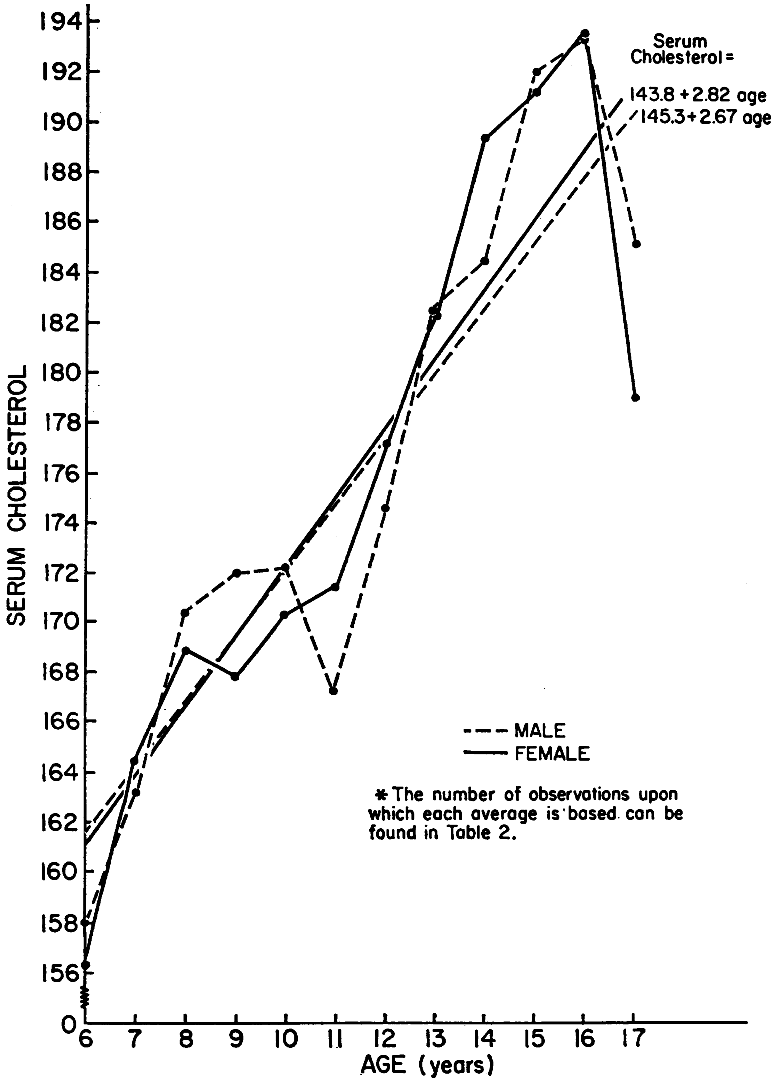
Beta Ratios

The examination of the beta ratios presents an entirely different picture as shown in Table 3.

Table 2—Average Serum Cholesterol Values Among 1,321 School Children by Age, Race, and Sex

Age (in Years)	White Male		White Female		Nonwhite Male		Nonwhite Female	
	Number	Average	Number	Average	Number	Average	Number	Average
6	31	158.0	24	156.2	9	137.4	11	137.4
7	34	163.2	43	164.4	21	151.1	31	153.8
8	33	170.4	33	168.9	15	157.1	24	159.4
9	25	172.0	17	167.8	22	173.4	13	173.2
10	43	172.3	26	170.3	21	172.8	19	177.4
11	32	176.3	40	171.4	19	183.6	19	177.8
12	46	174.6	36	177.1	27	177.2	24	182.5
13	35	183.7	34	182.3	20	175.4	26	186.6
14	53	184.6	40	189.4	18	175.3	18	176.6
15	32	192.0	34	191.2	20	184.5	23	196.2
16	38	193.3	30	193.6	15	188.5	27	188.9
17	27	184.8	31	179.1	12	182.8	12	203.6
18	8	179.1	5	190.8	6	183.7	7	196.7
19	4	176.0	—	—	1	180.0	—	—
20	3	198.0	—	—	3	210.0	1	190.0
Total	444	177.0	393	176.8	229	173.7	255	177.0
Average increase per year	2.7		2.8		3.3		4.3	
Average age in years	11.87		11.71		11.81		11.80	

Figure 1—Average* Serum Cholesterol Levels Among White Children in Evans County, by Age and Sex



The striking observation from the study of the beta ratios is the consistently and significantly greater values found among the white children. This differential is fairly constant over all ages studied here and for both sexes and amounts to approximately 4.5 percentage units.

In contrast to the cholesterol data, there is no growth trend except for the peculiar spurt among white male children from ages 10 to 11. Prior to and including age ten, these children had a beta ratio consistently hovering around 70.0, whereas starting at age 11 and thereafter, the average beta ratio was consistently between 74.0 and 76.0 percentage points. This phenomenon might be related to pubertal development except that it was not seen among the nonwhite males nor were there any similar changes among the females associated with their sexual development.

There does not seem to be any consistent differential between the sexes with the exception of this peculiar interaction among white children. Prior to age 11, the white males are lower and subsequent to that age the reverse seems to hold true.

Serum Cholesterol and Beta Ratio

Owing to the fact that cholesterol was related to age but that beta ratios did not appear to be so associated (with the exception of white males), it would be expected that the correlation between cholesterol and beta ratio would be of a small order of magnitude. The largest correlation should occur among white males and this is exactly what happened.

The simple correlations between cholesterol and beta ratio are as follows:

	White Male	White Female	Non-white Male	Non-white Female
Correlation coefficient	0.23	0.14	0.06	0.18
Number of observations	414	376	220	250

Figure 2—Average* Serum Cholesterol Levels Among Nonwhite Children in Evans County, by Age and Sex

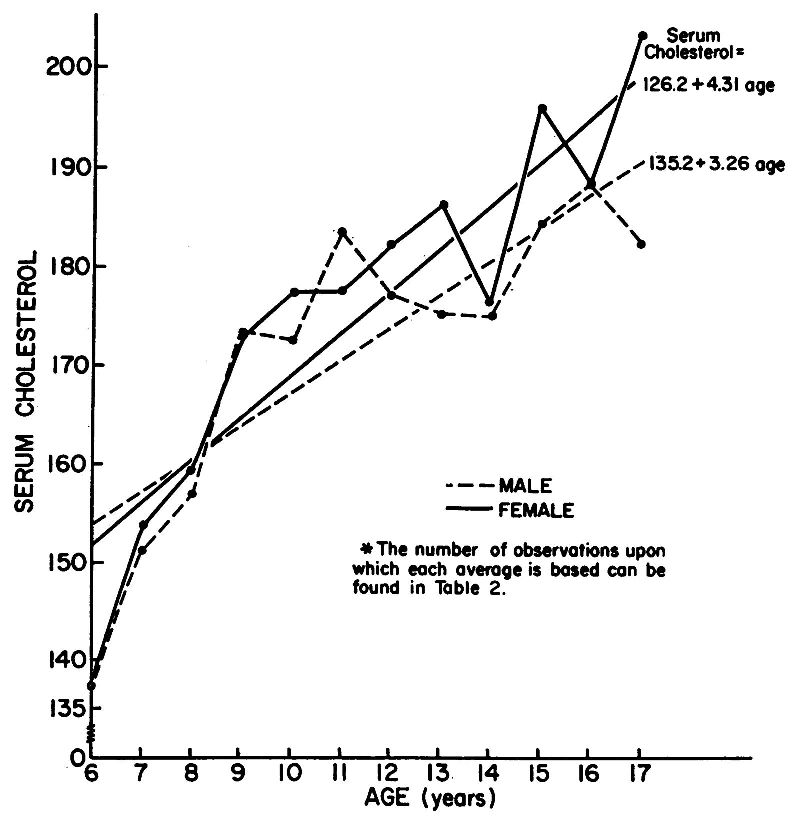


Table 3—Average Beta Ratios Among 1,263 School Children by Age, Race, and Sex

Age (in Years)	White Male		White Female		Nonwhite Male		Nonwhite Female	
	Number	Average	Number	Average	Number	Average	Number	Average
6	31	70.5	24	72.9	9	64.4	10	65.1
7	33	70.9	43	72.7	21	70.9	30	68.4
8	33	70.5	32	73.3	14	65.6	24	67.5
9	25	70.1	16	72.4	19	68.2	13	68.0
10	26	69.5	20	72.5	20	68.2	19	71.2
11	32	74.0	36	71.5	19	66.9	19	72.7
12	44	74.2	36	74.9	24	67.3	24	69.3
13	31	74.5	34	70.2	20	68.4	26	67.8
14	51	73.2	40	69.4	18	67.4	17	68.1
15	32	74.2	31	73.4	19	68.7	23	66.3
16	38	76.3	29	74.7	15	65.2	26	70.4
17	26	76.1	30	73.8	12	69.8	12	71.1
18	7	75.8	5	69.0	6	69.8	7	68.3
19	4	76.8	—	—	1	78.0	—	—
20	3	78.7	—	—	3	74.3	1	59.0
Total	416	73.18	376	72.51	220	67.98	251	68.81
Average age	11.91		11.71		11.86		11.76	

These correlation coefficients, except for the nonwhite males, show a statistically significant association between the two sets of measurements. The association is, of course, a slight one since the magnitude of the correlation coefficient is not very great.

Other studies in Evans County of serum cholesterol and beta ratios, when coupled with the previous relationship, make an interesting observation. Using fetal cord blood from newborn infants, there was no association between cholesterol and beta ratios at birth. Among the mothers who gave birth to these infants, the correlation between these two variables was significantly different from zero and of a larger order of magnitude than that found among the present school children. Thus, it seems that the serum cholesterol and beta ratio are independent at birth but become more strongly associated with each other as the individual ages.

Further Analysis Using Height, Weight, and Blood Type

Detailed analysis of the 1,402 observations has not yet been made upon the relationship of serum cholesterol and beta ratio to height, weight, and blood type. An earlier examination of the first three grades of school, however, is presented here to point out interesting aspects. In the first three grades of school, there were 418 observations on serum cholesterol and 394 on beta ratio.

A. Influence of Height and Weight—(1) To determine whether taller children have higher or lower serum cholesterol and beta ratio values, correlation coefficients were used to measure the strength of this relationship. In as much as a child's height is a function of his age, when these coefficients are adjusted for age differences, the result is called a partial correlation coefficient.

In Table 4, these partial correlation coefficients are seen to differ very little from zero, and show no discernible trend for either race or sex. This means that those factors which are considered growth determinants, viz. heredity, hormones, diet, disease, and other environmental influences, do not have a combined influence upon either serum cholesterol or beta ratio during the ages from 6 to 11.

(2) To determine whether those factors which influence weight have an influence upon serum lipids, a similar analysis was performed by substituting weight for height. The weight of a child, however, is not only a function of his age but also his height. Therefore, the partial correlation coefficients were adjusted for both age and height. The result is that the comparison is intended to measure the relationship of serum cholesterol and beta ratio with the stockiness of the child.

Does the stocky or heavy child tend to have a higher or lower cholesterol? In Table 5, the partial correlation coefficients are seen to be quite small again in their magnitude. An interesting difference between this table and the preceding one, however, is that there appears for the females to be a slight association between stockiness and serum cholesterol, and between stockiness and beta ratio. That is, for girls between the ages of from 6 to 11, there appears to be a slight but significant relationship between the stockiness of the girl and serum cholesterol and beta ratio. For boys, the relationship is not so evident and is even possibly reversed for the beta ratio.

B. Influence of Blood Type—The relationships between blood type (O, A, B, AB) and serum cholesterol or beta ratio are of interest to determine whether the latter measurements are in some way linked to the genetic composition of the individual.

Separate analyses were performed on the white and nonwhite children since race is definitely associated with blood type. Sex was ignored and the data pooled since there is no evidence that blood types differ by sex.

The beta ratios were analyzed by an analysis of covariance to adjust for age and no differences were found in either race. The serum cholesterol, on the other hand, produced interesting findings for both white and nonwhite children. In Table 6, it can be seen that the average cholesterol values, adjusted for age differences, differ in the various blood groups. The adjustment for age differences was necessary because each blood group did not contain exactly the same age distribution of children. The analysis of covariance is shown in Table 7.

Table 4—Partial Correlation Coefficients of Serum Cholesterol and Beta Ratio with Height Adjusted for Age, by Race and Sex

Group	Serum Cholesterol		Beta Ratio	
	Number of Cases	Coefficient	Number of Cases	Coefficient
White male	126	0.09	120	0.02
White female	113	0.09	102	−0.08
Nonwhite male	91	−0.05	85	0.04
Nonwhite female	90	0.08	87	0.09

Table 5—Partial Correlation Coefficients of Serum Cholesterol and Beta Ratio with Weight Adjusted for Age and Height, by Race and Sex

Group	Serum Cholesterol		Beta Ratio	
	Number of Cases	Coefficient	Number of Cases	Coefficient
White male	126	0.00	120	−0.05
White female	113	0.16	102	0.17
Nonwhite male	91	0.05	85	−0.13
Nonwhite female	90	0.08	87	0.13

Table 6—Average Serum Cholesterol Values of Children in First Three Grades of School Adjusted for Age, by Blood Group, and Race

Blood Type	White		Nonwhite	
	Number of Cases	Serum Cholesterol	Number of Cases	Serum Cholesterol
O	113	167.7	102	162.1
A	81	163.7	39	158.8
B	15	159.4	37	154.5
AB	5	165.2	2	——*

* Number of cases too small to assign a reliable value here.

The consistency of the differences for both races as shown in Table 6, about 4.0 units each in going from O to A to B was of special merit. The differences were significant at the 5 per cent probability level after adjusting to an average age of 8¼ years in the analysis of covariance.

The complete analysis of the 1,402 observations with respect to blood type is under investigation using the same method. The unadjusted values of average serum cholesterol are not as widely divergent among the four blood groups as they appeared to be with the first three grades of school. One point which was definitely repeated in both races, however, was the low serum cholesterol values for those in blood type B. The difference amounted to about 5 mg per cent.

Discussion

The present data, in conjunction with observations made by other investigators, raise the question of the nature of the relationship between serum cholesterol and beta lipoprotein to atherogenesis.

The very nature of atherosclerosis makes this a most difficult relationship to elucidate because of these reasons alone: (1) The mechanism by which atherosclerosis occurs is not known; (2) the extent of its occurrence cannot be determined during life; (3) the extent of its involvement does not always parallel its clinical manifestations. There may be minimal atherosclerosis in a critical area of the cardiovascular system with thrombosis and death in contrast to widespread, severe atherosclerosis with no clinical symptoms. This paradox of apparent health in the presence of severe disease makes it extremely difficult to define adequately normal controls, to study the incidence or prevalence, and to measure the progression, therapy, or prevention of the disease. In fact, the whole concept of relating lipids to atherogenesis in man is still based on association and inference. At present, there is no direct evidence which will allow the transfer of data gained from experimental animals to man.

Holman[4] has divided the natural history of the disease into roughly four stages: fatty streak, ages from 0 to 19; fibrous plaque, ages 20-39; complication of lesions; and clinical disease over 40. He measured the percentage of total area involved with fatty streaks on the inner surface of the aorta, which stained red with sudan IV in all chronological age groups from birth to old age. This study demonstrated the universal occurrence of the fatty streak which he considered the precursor of atherosclerosis in all individuals over three years of age. Of the total aortic area involvement with fatty streaks which develops during the life span, almost the maximum has occurred before age 20. This phenomenon has been demonstrated in pathological samples from eight widely separated geographic areas of the world. It is unlikely that the Evans County population would be very different.

In an unpublished study of almost 2,000 cholesterol determinations in individuals in Evans County under age 18, fetal cord blood levels averaged about 80 mg per cent and gradually increased to a little less than 180 mg per cent by age 18. Here we have an interesting spectacle. Progressive development to almost maximum area involvement of aortic fatty streaks at a time when cholesterol levels were well below 200 mg per cent!

Such an orientation suggests many vital questions. What is the relationship of serum cholesterol to atherogenesis? Does lowering serum cholesterol to levels that are compatible with the American way of life actually retard atherogenesis since we know there has been progressive development of the fatty streak in school children with levels well below 180 mg per cent?

In Holman's study, the nonwhite age group (11-15 years) showed approxi-

Table 7—Analysis of Covariance of Serum Cholesterol for Nonwhite School Children, by Blood Type, Adjusted for Age Differences

Source of Variation	Degrees of Freedom	Sum of Squares for Age	Sum of Products for Age, Cholesterol	Sum of Squares for Cholesterol	Adjusted Sum of Squares for Cholesterol	Degrees of Freedom	Mean Squares
Between blood types	3	1.4184	54.5455	3,256.1246			
Within blood types	176	481.3094	3,346.7156	84,040.8698	60,769.9648	175	347.26
Total	179	482.7278	3,401.2611	87,296.9944	63,331.9833	178	
Between blood types					2,562.0185	3	854.01

$F = \frac{854.01}{347.26} = 2.46$ significant at 5 per cent.

mately four times more fatty streaks than presumably comparable white age groups. In this survey in Evans County, the serum cholesterol levels were approximately equal during this age period. This fact alone, assuming Evans County's population is no different from other geographic areas studied, would tend to suggest the lack of a simple direct relationship between the occurrence of fatty streaks and serum cholesterol levels per se. On the other hand, one might reflect about the possible relationship between the increased fatty streaks among nonwhites (11-15) observed by Holman, and the present observation that the nonwhites from 6 to 9 years of age experienced a marked increase in serum cholesterol from levels which had previously been lower than whites. The rate of increase may be the key factor.

Summary

In a study of approximately 1,400 school children, aged from 6 to 20 years, in Evans County, Ga., the following was observed:

1. Beta ratios did not vary with age except for white males who exhibited a sudden spurt in going from 10 to 11 years of age.
2. Beta ratios appeared to be higher in the white than the nonwhite. There was no sex difference in the nonwhite groups but the white males aged from 6 to 10 years were definitely lower than the females and about the same thereafter.
3. Whites had significantly higher cholesterol levels in ages from 6 to 8. After that age, no race difference occurred. Cholesterol levels rose about 2.75 mg per cent per year for whites and about 3.8 mg per cent for nonwhites.
4. The rate of increase in cholesterol, level was faster among the nonwhites, particularly at the younger ages.
5. There was a slight association between stockiness and serum cholesterol for both white and nonwhite females.
6. Analysis of the observations from the first three grades of school with respect to blood type showed cholesterol levels varying with blood type. Preliminary analysis of all 12 grades of observations confirmed the low serum cholesterol values for those persons in blood type B. The difference amounted to about 5 mg per cent.
7. The very early universal appearance of fatty streaks and their progressive evolvement (as observed in other studies) at a time when serum cholesterol levels are well below 200 mg per cent, suggests a need for reevaluation of the present widely accepted concept of a direct causal relationship between serum lipid levels and arterial wall lipid accumulation that is commonly assumed to be the first stage of atherosclerosis.

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ADDENDUM

(1) The comparison of the levels of serum cholesterol according to blood group, for each race, has been extended to include the children of all ages in the study. The findings for all ages do not confirm the differences observed among the first three grades of school children. Although the differentials between O and A and between A and B groups are in the same direction for the complete sample as those observed in the smaller subsample, the magnitude of each is reduced and statistically nonsignificant.

This change might be caused by the fact that as children grow older, environmental influences tend to dilute any genetic differential observed at earlier ages. On the other hand, the disappearance of an apparent genetic difference in this case might be statistical artifact because the study was cross-sectional in nature whereas the data have been analyzed as if they had been longitudinal. The apparent diminution of the difference with age might be a cohort factor. That is, the young children, as they themselves grow older, might continue to display the same difference among blood groups as they did at earlier ages.

(2) Cholesterol determinations in this laboratory are now by the Abell-Kendall method. Results have been standardized with Dr. Jeremiah Stamler's laboratory, Heart Disease Control Program, Chicago Board of Health. Because of recent reports in the literature questioning the validity of the Zlatkis, Zak, and Boyle method and since the serum specimens had been frozen and were available, repeat determinations were made on a representative sample using the Abell-Kendall method. The re-run of these samples confirmed the previous findings and no final conclusions were thereby affected.

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This paper was presented before the Statistics Section of the American Public Health Association at the Eighty-Seventh Annual Meeting in Atlantic City, N. J., October 22, 1959.

This investigation was supported (in part) by a grant (H-3341) from the National Heart Institute, U. S. Public Health Service, Washington, D. C.

Continuity of Care Is of Paramount Importance

"The number and complexity of the professional disciplines which may be properly directed toward the rehabilitation of even a single individual is equaled only by the number of institutions and agencies which can become involved with the problem on a community level. Just as properly oriented and trained medical direction is necessary in coordinating the many disciplines involved in the care of a single patient, so is properly oriented and trained direction indispensable for the best development and utilization of the many community facilities and agencies. Moreover, with the interest of the total person in mind, continuity of care becomes of paramount importance. This applies both to the groups involved in the medical treatment of the patient and the agencies involved in the socioeconomic and other community aspects of his problems.

"It is the responsibility of the health department to develop an awareness of this basic need for a unified approach to total patient consideration in rehabilitation and its corollary, the continuity of care, through all the successive phases involved in such activities." (Milton Feig, M.D., M.P.H., in October-December, 1960, issue of Health, quarterly bulletin of the Wisconsin State Board of Health.)