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Cholesterol Ester and Phospholipid Composition of Normal Aortas and of Atherosclerotic Lesions in Children[1]

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Gas chromatographic techniques have been used to study the lipid composition of children's arteries over the first decade of life. Children's aortas were separated into intima and media and, where present, into intimal fatty streak lesions and media underlying such lesions, and the level of cholesterol ester and of phospholipid and the fatty acid composition of these fractions in these various portions of the aortas was determined. The ester cholesterol concentration of the normal intima, but not of the media, increased with age. The ester cholesterol concentration of the fatty streak lesions exceeded that of the normal intima and also increased with age. No changes with age in phospholipid content for normal intima or media or for the intimal lesion were observed. The cholesterol ester fatty acid composition of the normal intima and of the media was similar to that of the lesions studied, and all tissues manifested a change in composition with age; cholesterol linoleate increasing and cholesterol palmitate decreasing during the first decade. These age changes for the aortic fractions were shown to resemble changes with age in the serum cholesterol ester fatty acids. The phospholipid fatty acid pattern of normal intima and media resembled those of the lesion but no change with age in phospholipid fatty acid pattern was found. No correspondence of the aortic phospholipid pattern with the serum pattern was demonstrated. These data are consistent with the view that arterial cholesterol ester, but not phospholipid, in both the normal intima and in the early fatty streak lesion in children arises initially from serum.

The analysis by gas–liquid chromatography of the fatty acid composition of the cholesterol esters of adult arteries has yielded information which indicates that the cholesterol esters which accumulate in the normal intima may arise by infiltration from the blood (Smith, 1962, 1965). The fatty streak type of atherosclerotic lesion, however, has a cholesterol ester composition which is quite distinct from that of the serum in possessing a higher proportion of cholesterol oleate and a lower proportion of cholesterol linoleate (Luddy *et al.*, 1958; Swell *et al.*, 1960; Geer and Guidry, 1964; Smith, 1965). The suggestion has been made, therefore, that the deposition of cholesterol ester in the early lesion may be contributed to either by synthesis in the wall or by differential removal of the more polyunsaturated cholesterol linoleate. Early fatty streak lesions occur, however, in young children (Zeek, 1930; Holman *et al.*, 1958; Zugibe and Brown, 1960; Van Belle *et al.*, 1964; Schwartz *et al.*, 1967) and there have been reports which suggest that normal arteries from young children

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contain no cholesterol ester (Tuna and Mangold, 1963; Scott *et al.*, 1966). The investigation of the type of cholesterol esters which are deposited in the normal intima of young children with advancing age and the type of cholesterol ester present in these very early fatty streak lesions would be of interest, therefore, in determining the factors associated with the earliest accumulation of this lipid fraction in the atherosclerotic artery. Some information regarding the lipid composition of children's arteries is available (Meyer *et al.*, 1966; Scott *et al.*, 1966; Wiese *et al.*, 1967), but no attempt has been made to determine the cholesterol ester or phospholipid fatty acid composition of the normal intima and of the lesion in such material. This is understandable since the intima in childhood is extremely thin. Nevertheless, by suitable dissection an inner layer can be obtained and in the present paper this approach has been employed to study the concentration of cholesterol ester and phospholipid and their fatty acid compositions in both normal intima and normal media and also in fatty streak lesions, of aortas obtained postmortem from infants and children.

MATERIALS AND METHODS

Arteries

The ascending aorta or aortic arch was obtained postmortem from 56 children (aged from a few hours to 10 years). Eleven of these had macroscopically visible raised yellow or white–yellow areas usually just above the aortic valves or adjacent to the origin of the vessels. The common causes of death were congenital abnormalities, respiratory disease, neoplasia, and trauma; arteries from patients with known metabolic disorders were excluded from the series. The aortas were deep-frozen on removal. They were subsequently thawed, adherent fat removed from the adventitial side, and washed several times in 0.9% sodium chloride solution over a period of about 30 minutes to remove traces of serum. Where atherosclerotic lesions were macroscopically visible, these were dissected from surrounding normal tissue with the aid of a dissecting microscope (×10.5 magnification). Intima and as little media as possible were stripped from the underlying media and adventitia with the aid of fine dissecting instruments. The stripping technique was checked histologically and found to provide, for normal intima, an intima/media preparation of about 1/10 the overall wall thickness (Fig. 1). In view of the small amount of cholesterol ester present in the age groups under 1 year it was found necessary to pool respectively aortic intima, media, or lesions (where present) from five children. These were collected into chloroform:methanol 2:1 for final processing as a group. No macroscopic lesions were present, however, in the aortas from children under 1 month, so that no information on lesions in this age group can be presented. For the 15 aortas studied in the 1-month to 1-year age group, macroscopic lesions were present in 6. These lesions were combined for extraction into one of three groups corresponding to their adjacent normal intima. The three lipid extracts of the lesions of this age group, therefore, contained material from six aortas and not from 15 as was the case for the normal intima. Tissues from older children were processed individually. Lipid extracts were prepared by the method of Folch *et al.* (1957). All solvents used were redistilled and prechecked by methyl esterification and gas–liquid chromatog-

FIG. 1. Aorta from a child aged 7 years 4 months showing thin inner layer, consisting of intima with some media, stripped from the underlying media. Hematoxylin and Sudan IV. ×40.

raphy as described below. Instruments used were cleaned immediately before use with lipid-free solvents. Blanks were run throughout all steps and with each batch of samples. Defatted tissues were dried to constant weight to allow the expression of lipid content in relation to dried defatted weight. These weights ranged between 15 and 180 mg for the intima (although in one case the lesion intima was only 5.5 mg) and between 50 and 2000 mg for the media.

Determination of lipid content

Cholesterol ester. In order to determine the small amount of cholesterol ester in the aortic tissues, cholesterol heptadecanoate was added as an internal standard to the lipid extracts. Cholesterol esters were separated by thin-layer chromatography as set out below. Cholesterol ester values were then obtained by gas–liquid chromatography by comparison with the internal standard. Thus, not only the cholesterol ester content, but also the fatty acid composition of the cholesterol ester could be determined. The cholesterol heptadecanoate was synthesized chemically via the acyl chloride by the method of Pinter *et al.* (1964). Heptadecanoic acid was obtained from the Hormel Institute, Austin, Minnesota, and cholesterol USP from Nutritional Biochemical Corporation, Cleveland, Ohio. Purification of the cholesterol heptadecanoate, after initial separation of the cholesterol esters on silicic acid, was achieved

by thin-layer chromatography on silver nitrate-impregnated silicic acid according to the method of Morris (1964). Cholesterol esters were visualized using a spray of 0.2% dichlorofluorescein in ethanol and the cholesterol heptadecanoate scraped from the plate and eluted with chloroform: petroleum ether 9:1. Fatty acid purity was checked by gas–liquid chromatography and only one peak, corresponding to heptadecanoic acid, was found. The final product was assayed for cholesterol by the method of Zlatkis *et al.* (1953) after saponification by the method of Abell *et al.* (1952). It was evaluated as an internal standard by assaying against a known amount of cholesterol stearate (Applied Science Laboratories, State College, Pennsylvania). The coefficient of variation ($n = 4$) was 1.1%. The colorimetric and internal standard methods agreed to within 7%. The cholesterol ester in the aortic extracts was expressed as micrograms of cholesterol/100 mg dry defatted tissue.

Free cholesterol. ^{14}C-labeled cholesterol (55.8 mCi/mmole, Radiochemical Centre, Amersham, U.K.) was added to the aortic lipid extract as an internal standard to allow determination of recovery. The free cholesterol was then obtained after separation of the aortic lipid extract by thin-layer chromatography as set out below. Cholesterol was eluted with 2:1 chloroform: methanol. The eluant was made up to a known volume, aliquots being taken for counting using the dioxane–water scintillator of Snyder (1964) in a Packard TriCarb Spectrometer Model 3375. Further aliquots were taken for cholesterol determination by the method of Zlatkis *et al.* (1953). As indicated by the ^{14}C-labeled cholesterol internal standard, between 80 and 90% recovery was achieved, but correction for recovery was made in calculating the amount of free cholesterol present in the aortic tissue.

Phospholipid. After separation of the aortic extracts by thin-layer chromatography, phospholipid was eluted by the method of Arvidson (1967). Recoveries were found to be in excess of 90% as determined using ^{14}C-labeled arterial phosphlipid. However, this was not applied as a correction factor as the ^{14}C was not included in the sample as an internal standard. The eluate was made up to a known volume, and aliquots were taken for determination of lipid phosphorus by the method of Bartlett (1959) and for determination of its fatty acid composition after methyl esterification by gas–liquid chromatography.

Sera. Serum was obtained from umbilical cord blood, from children and from adults. An aliquot of each sample was extracted by the method of Folch *et al.* (1957) Phospholipid and cholesterol ester were separated by thin-layer chromatography and their fatty acid composition determined by gas–liquid chromatography after methyl esterification as set out below.

Thin-layer chromatography. Phospholipid, cholesterol, triglyceride, and cholesterol ester were separated by thin-layer chromatography on Silica Gel G (Merck) using diethyl ether: acetic acid: *n*-hexane in the proportions 125:38:3 v/v/v.

Gas–liquid chromatography. Cholesterol ester and phospholipid were separated by thin-layer chromatography and their fatty acid methyl esters obtained by heating at 65°C with 5% sulfuric acid in methanol in sealed ampules and subsequent extraction with petroleum ether. The methyl esters were run on columns of diethylene glycol succinate on gas chrom P (Applied Science) at 185°C in an F & M Model 5750 gas chromatograph. Identification of peaks was either on the basis of retention

time relative to methyl palmitate or, for 20:3 ω 6 and 20:3 ω 9, relative to 20:4 (RT of 0.9 and 0.8, respectively, as determined with the aid of phospholipid from essential fatty acid-deficient rats, supplied by courtesy of Dr. F. D. Collins, Department of Biochemistry, University of Melbourne). For each methyl ester the product of peak height and retention time was calculated and this information used to derive the percentage chemical distribution. Quantitative results with fatty acid standards KA, KB, KC, and KD from Applied Science Laboratories, State College, Pennsylvania, agreed with the stated comparison data with a relative error less than 5% for major components (> 10% of total mixture) and less than 10% for minor components (< 10% of total mixture). For blanks cholesterol heptadecanoate was also used as an internal standard and it was found that they contained 5–15% of the material in the arterial cholesterol ester runs. Phospholipid blanks were reckoned to contain less than 2% of the material in the samples.

RESULTS

Histological examination of macroscopically normal children's aortas revealed little sudanophilia. However, occasionally, apparently extracellular lipid droplets were seen in the inner arterial wall as was lipid associated with cells and sudanophilic smudging along the endothelial surface. Of the 56 proximal aortic specimens processed, 11 had visible fatty streaking. When possible, a portion of the small amount of abnormal aorta was processed for histology. Both extracellular and intracellular lipid was present in those lesions examined.

The amounts of cholesterol ester present in the normal intima and media and in the early fatty streak lesions present in the arteries are given in Table I. The amount of ester cholesterol present in the normal intima below 1 year is extremely small (10–20 μg/100 mg dry defatted tissue). However, this increases with age to approximately 150 μg/100 mg dry defatted weight at age 10 years. There is a significant positive correlation with age as shown by the correlation coefficient given. The amount of cholesterol ester in the lesion is appreciably higher than that in the corresponding normal intima. However, the number of lesions studied was not sufficient to demonstrate a significant positive correlation with age. The amount of cholesterol ester present in the media is similar to that in the intima for the younger age groups, but the increase with age is not marked, and no significant correlation with age could be demonstrated. The amount of ester cholesterol in the media underlying the lesion is not much greater than that in the surrounding media, and no change with age is apparent.

The amount of free cholesterol and the percentage of the total cholesterol present as ester in the four types of samples have also been given in Table I. The amount of free cholesterol present in each of the samples, normal intima, lesion, normal media, and media underlying the lesion, is considerably higher than that of the ester cholesterol. In the normal intima, the percentage of ester accounts for 4.5–15% of the total cholesterol. In the lesion, however, the ester cholesterol accounts for up to 35% of the total cholesterol present so that the cholesterol ester has apparently accumulated in relation to free cholesterol at a greater rate in the lesion than in the corresponding normal intima. The amount of free cholesterol in the normal intima rises with age so that at 5–10 years over 1 mg/100 mg dry defatted

TABLE I

CHOLESTEROL AND CHOLESTEROL ESTER CONTENT OF CHILDREN'S ARTERIES
(μg/100 mg dry defatted tissue)[a]

	Intima						Media					
	Normal			Lesion			Normal			Lesion		
	Free cholesterol	Ester cholesterol[b]	% Total cholesterol as ester	Free cholesterol	Ester cholesterol[b]	% Total cholesterol as ester	Free cholesterol	Ester cholesterol[b]	% Total cholesterol as ester	Free cholesterol	Ester cholesterol[b]	% Total cholesterol as ester
0–1 month[c]	350.9 ±57.1 (3)	16.7 ±0.3	4.5 ±0.3				282.0 ±84.5 (3)	16.4 ±5.7	5.7 ±1.8			
1 month–1 year[c]	279.2 ±45.7 (3)	39.9 ±8.9	12.3 ±0.6	332.9 ±88.9 (3)	114.1 ±40.3	24.5 ±2.1	248.0 ±62.7 (3)	19.6 ±7.3	7.0 ±1.5	204.8 ±88.1 (3)	35.8 ±20.7	14.0 ±3.0
1–5 years	497.8 ±229.9 (4)	47.0 ±20.9	10.3 ±4.3	1140.0 (1)	286.5	20.1	563.6 ±341.2 (3)	116.6 ±103.6	10.6 ±5.0	1246.0 (1)	323.8	20.6
5–10 years	1159.5 ±415.7 (5)	127.0 ±27.5	15.0 ±4.5	641.9 ±224.1 (2)	400.5 ±231.5	35.5 ±6.7	275.5 ±53.3 (5)	43.4 ±30.4	10.8 ±5.3	351.6 ±60.5 (3)	45.3 ±11.6	11.1 ±1.2
Correlation coefficient[d]	0.5966 (15) <.05	0.7480 <.001	0.3606 NS	0.4898 (6) NS	0.7445 NS	0.6976 NS	0.1570 (14) NS	0.4957 NS	0.5983 <.05	0.1578 (7) NS	0.6741 NS	−0.3182 NS

[a] Means and standard errors of means are given; numbers of samples are shown in parentheses.

[b] Ester cholesterol is expressed as cholesterol, conversion from GLC data being made as though all cholesterol ester was cholesterol heptadecanoate.

[c] Each sample represents five pooled specimens for the normal intima. The lesions present in the five aortas in each of the three groups from one month to one year are included in the corresponding lesion samples (i.e., 6 of the total 15 in this age group).

[d] Correlation coefficients are calculated from the values of individual samples.

tissue is present. This increase with age of the free cholesterol is statistically significant with a correlation coefficient of 0.5966. The concentration of free cholesterol in the media is approximately the same as that in the intima (Table I), but the rather large variance precludes adequate statistical comparison. It will be noted that the percentage of ester cholesterol present in the area underlying the lesion is rather lower than that of the lesion itself.

The fatty acid compositions of the cholesterol ester of normal intima and of fatty streak lesion are shown in Table II. The data are grouped into four age groups and the figures represent percentage distribution of the fatty acids present. In the normal intima of the first two groups, that is, 0–1 month and 1 month–1 year, the predominant fatty acid present in the cholesterol ester is oleic acid. There is also an appreciable amount of palmitic acid present but rather low amounts of linoleic acid. In the older age groups the amount of oleic acid is similar to that for the younger groups and still represents the predominant fatty acid at age 10 years. However, the amount of linoleic acid increases from 9.5 % at 0–1 month to 26.5 % at 5–10 years, an increase with age which is highly significant statistically. The percentage of palmitic acid decreases with age, so that by 5–10 years it has fallen to 17.4 % demonstrating a significant negative correlation with age.

The fatty acid patterns for the lesions are shown in the second part of Table II. Although the numbers are not as large as for the normal intima, it can be seen that the pattern is similar to that for the normal intima in the corresponding age group. The linoleic acid which is 11.4 % below 1 year has risen by 10 years in just the same way as for the normal intima.

The cholesterol ester fatty acid pattern of the normal media and of the media underlying the lesion is shown in Table III. The fatty acid pattern is almost identical with that of the intima, both with respect to the patterns at the various age groups and with respect to the increase with age of the linoleic acid and the fall with age of the palmitic acid. In the media underlying the lesions, a similar cholesterol ester fatty acid pattern is present and a similar age shift occurs, although the numbers do not enable a satisfactory statistical comparison to be made.

The cholesterol ester fatty acid patterns of the serum, studied in age groups similar to those for aorta are shown in Table IV. In umbilical cord serum and in serum under 1 year of age, the predominant cholesterol ester fatty acid is oleic acid and there is a relatively small proportion of linoleic acid. However, the oleic acid proportion falls somewhat with age and the linoleic acid proportion increases appreciably with age so that in the older groups linoleic acid is the major fatty acid. There is a reduction in the proportion of palmitic, palmitoleic, and arachidonic acid in serum cholesterol ester with age, age shifts which are statistically significant as indicated in the table. Comparison of the data for serum with that for intima (Tables II and IV) reveals a definite similarity in both the cholesterol ester fatty acid pattern for a given age group and in that pattern's change with age. In both intima and serum the linoleic acid rises and the palmitic acid falls with advancing age.

The phospholipid content in the various areas of the arteries studied is shown in Table V. There is considerably more phospholipid than cholesterol ester, but there is no significant difference between the amount of phospholipid present in normal

TABLE II

INTIMAL CHOLESTEROL ESTER FATTY ACID PATTERNS
(% distribution)[a]

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:3 ω 6	20:4	18:1/ 18:2
Normal										
0–1 month[b] (5)	1.2 ±1.1	29.4 ±3.1	11.0 ±2.2	7.3 ±1.7	36.6 ±2.9	9.5 ±2.3	5.2 ±2.2	0	0	4.90 ±1.23
1 month–1 year[b] (4)	1.1 ±1.1	20.3 ±4.7	10.6 ±0.9	4.2 ±1.2	42.0 ±3.7	12.9 ±2.8	2.1 ±1.2	0	1.1 ±1.1	4.23 ±0.90
1–5 years (5)	0.3 ±0.3	19.5 ±1.5	5.6 ±0.5	7.5 ±2.0	40.2 ±2.6	20.9 ±3.8	0	0	6.2 ±1.5	2.39 ±0.068
5–10 years (6)	0.6 ±0.6	17.4 ±2.0	7.5 ±1.2	5.1 ±0.9	37.1 ±2.2	26.5 ±4.0	0.2 ±0.2	0	5.5 ±1.1	1.59 ±0.28
Correlation coefficient[c] (20)		−0.5031	−0.3592		−0.2317	0.6939			0.6345	−0.5902
p		<0.05	NS		NS	<.001			.01	<.01
Lesion										
1 month–1 year[d] (3)	0.8 ±0.8	18.5 ±1.6	6.9 ±1.0	5.8 ±1.4	50.9 ±2.3	11.4 ±1.3	4.1 ±2.1	0	1.7 ±1.7	4.62 ±0.67
1–5 years (1)	0	20.2	6.3	7.1	32.4	24.5	0	0	9.8	1.32
5–10 years (4)	2.0 ±2.0	18.9 ±5.7	6.8 ±1.8	4.5 ±2.4	35.4 ±4.7	25.8 ±6.6	0.5 ±0.5	0.7 ±0.7	5.3 ±1.3	1.52 ±0.28
Correlation coefficient[c] (8)		−0.1880	−0.2590		−0.6371	0.8485			0.5885	−0.8565
p		NS	NS		NS	<.05			NS	<.05

[a] Means and standard errors of means are given; numbers of samples are shown in parentheses.

[b] Each sample represents five pooled specimens.

[c] Correlation coefficients are calculated from the values of individual samples.

[d] The lesions present in the five aortas in each of the three groups are included in the corresponding lesion samples (i.e., from 6 aortas out of a total of 15 aortas in this age group).

TABLE III

MEDIAL CHOLESTEROL ESTER FATTY ACID PATTERNS
(% distribution)[a]

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:3 ω 6	20:4	18:1/ 18:2
Normal										
0–1 month[b] (5)	0.8 ±0.5	23.4 ±1.7	11.4 ±1.3	3.5 ±0.2	39.9 ±1.0	11.8 ±2.2	0 ±0	0 ±0	9.3 ±1.6	3.92 ±0.75
1 month–1 year[b] (3)	0.7 ±0.7	19.0 ±1.3	9.8 ±1.0	3.6 ±0.7	48.8 ±4.0	14.1 ±2.3	0	0	4.1 ±0.6	3.76 ±0.93
1–5 years (5)	0.5 ±0.5	24.3 ±1.7	6.8 ±1.8	9.4 ±1.0	41.0 ±3.8	13.9 ±4.0	0	0	4.4 ±1.0	5.44 ±2.73
5–10 years (6)	0.3 ±0.3	17.2 ±1.0	6.3 ±0.3	5.4 ±1.7	37.4 ±1.3	27.5 ±3.0	0.1 ±0.1	0	6.2 ±0.9	1.47 ±0.21
Correlation coefficient[c] (19)		−0.4915 <.05	−0.5044 <.05		0.3824 NS	0.6692 <.01			−0.1757 NS	−0.3346 NS
Lesion										
1 month–1 year[d] (3)	0	22.9 ±9.4	6.5 ±0.5	5.5 ±1.3	49.2 ±2.1	11.9 ±2.9	0	0	4.3 ±0.5	4.60 ±1.03
1–5 years (1)	0	15.3	5.4	3.6	34.1	32.7	0	0	9.3	1.04
5–10 years (4)	0.8 ±0.7	17.4 ±1.1	6.0 ±0.5	4.5 ±0.6	38.7 ±2.6	28.3 ±2.9	0.1 ±0.1	0.4 ±0.4	4.3 ±1.0	1.45 ±0.26
Correlation coefficient[c] (8)		−0.4623	−0.2882		−0.5618	0.6943			−0.0590	−0.7127
p		NS	NS		NS	NS			NS	NS

[a] Means and standard errors of means are given; number of samples are shown in parentheses.

[b] Each sample represents five pooled specimens.

[c] Correlation coefficients are calculated from the values of individual samples.

[d] The lesions present in the five aortas in each of the three groups are included in the corresponding lesion samples (i.e., from 6 aortas out of a total of 15 aortas in this age group).

TABLE IV

SERUM CHOLESTEROL ESTER FATTY ACID PATTERNS
(% Distribution)[a]

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:3ω9	20:3ω6	20:4	18:1/18:2
Umbilical (10)	0 ± 0	20.0 ±0.5	11.3 ±0.6	3.6 ±0.3	40.3 ±1.9	13.2 ±1.0	0.2 ± 0.1	0 ±0	0 ± 0	0 ± 0	11.7 ±1.5	3.27 ±0.41
1 day–1 year (10)	1.1 ± 0.2	18.0 ±1.4	12.2 ±1.0	2.0 ±0.4	42.5 ±2.3	17.9 ±3.1	0.8 ± 0.1	0.7 ±0.2	0 ± 0	0 ± 0	5.7 ±0.9	3.24 ±0.60
1–5 years (17)	0.8 ± 0.1	14.2 ±0.7	6.0 ±0.4	2.0 ±0.2	35.2 ±1.4	35.1 ±2.4	1.1 ± 0.2	0.7 ±0.1	0 ± 0	0.1 ± 0.1	6.4 ±0.5	1.12 ±0.13
5–15 years (9)	0.9 ± 0.1	12.7 ±0.7	4.8 ±0.3	1.3 ±0.2	31.1 ±1.3	42.7 ±2.0	1.3 ± 0.2	0.7 ±0.2	0 ± 0	0.2 ± 0.1	5.4 ±0.6	0.75 ±0.07
15–30 years (9)	0.5 ± 0.0	12.0 ±0.3	4.7 ±0.6	1.3 ±0.1	31.9 ±1.5	43.8 ±1.9	1.0 ± 0.3	0.7 ±0.1	0 ± 0	0.1 ± 0.1	5.5 ±0.4	0.75 ±0.08
Correlation coefficient (55)[b]		−0.5161	−0.5605		−0.4292	0.6125					−0.2678	−0.4815
p		<.001	<.001		<.01	<.001					<.05	<.001

[a] Means and standard errors of means are given; numbers of samples are shown in parentheses.
[b] Correlation coefficients are calculated from the values of individual samples.

TABLE V

PHOSPHOLIPID CONTENT OF CHILDREN'S ARTERIES
(μg/100 mg dry defatted weight)[a]

	Intima		Media	
	Normal	Lesion	Normal	Lesion
0–1 month[b]	840 ± 252 (3)		1100 ± 318 (3)	
1 month–1 year[b]	1201 ± 1037 (3)	1765 ± 632 (3)	953 ± 360 (3)	1339 ± 844 (3)
1–5 years	1129 ± 211 (4)	909 (1)	718 ± 368 (4)	280 (1)
5–10 years	1310 ± 505 (5)	1148 ± 244 (3)	386 ± 113 (5)	784 ± 86 (3)
Correlation coefficient[c]	0.2282 (15)	−0.3593 (7)	−0.5309 (15)	−0.2749 (7)
p	NS	NS	<.05	NS

[a] Amount of phospholipid is calculated by multiplying × 25 the lipid P determined; means and standard errors of means are given. Number of samples are shown in parentheses.
[b] Each sample represents five pooled specimens for the normal intima. The lesions present in the five aortas in each of the three groups are included in the corresponding lesion samples (i.e., 6 out of the total 15 in this age group).
[c] Correlation coefficients are calculated from the values of individual samples.