

intima and in lesion, nor between that in normal media and media underlying lesion. Within the limitations of the numbers studied, there is also no evidence of any shift with age in the concentration of phospholipid, with the exception of normal media where there is a significant negative correlation with age. The amount of phospholipid is approximately the same, however, as that of the free cholesterol whereas it is considerably in excess of cholesterol ester.

The total phospholipid fatty acid pattern for the normal intima and for the intimal lesion is given in Table VI. Approximately equal proportions of palmitic, stearic, oleic, and arachidonic acid are present in these samples in both the normal intima and in the lesion. Unlike the change with age of the cholesterol ester fatty acids, there is no shift in any of these fatty acids with age in either the normal intima or in the intimal lesion. There is no appreciable amount of 20:3 ω 9 present in any of the samples, although 3-5% of the phospholipid fatty acids are 20:3 ω 6. While there are no significant changes in the major components with respect to age, there is a slight rise in the linoleic acid content of the lesion with age from 2.4 to 5.4%, an age trend which is statistically significant, and, in view of this change, the 18:1-18:2 ratio is also negatively correlated with age at a statistically significant level.

The medial phospholipid fatty acid patterns are shown in Table VII. Again approximately equal proportions of palmitic, oleic, stearic, and arachidonic acid are present in both the normal media and in the media underlying the lesion. The patterns are similar to those reported in Table VI for the intima and, with the exception of a fall in palmitic acid in the normal media, there is again no shift of the major four fatty acids with respect to age.

The serum phospholipid fatty acid patterns are given in Table VIII for the five groups studied. The proportions of stearic and oleic acid are rather smaller than in the corresponding age groups for the intima, but the most marked differences between the serum phospholipid fatty acid pattern and the intimal phospholipid fatty acid pattern are in their respective proportions of linoleic and arachidonic acid. The serum contains appreciably more linoleic acid in all age groups, but there is also a positive correlation with age of this component in the serum, so that the difference becomes more marked in the later age groups. The arachidonic acid also falls with age in the serum and again the difference between the serum and the intima with respect to its arachidonic acid content becomes more marked in the later age groups. Small amounts of 20:3 ω 9 are present in all of the age groups, but in the umbilical cord blood this represents over 1% of the total phospholipid fatty acid.

DISCUSSION

In the present study the intima, media, and the fatty streak lesion, have been observed separately and it has been possible to quantitate the amount of cholesterol ester in these fractions obtained from aortas in children over the first decade of life. The amount of cholesterol ester in the normal intima below 1 year is extremely small, representing only about 10-20 μ g/100 mg dry weight. The amount increases progressively with age, however, showing a positive correlation during the first decade. Both Smith (1965) and Insull and Bartsch (1966) have demonstrated that cholesterol ester accumulates in the normal intima over succeeding decades more

TABLE VI
 INTIMAL PHOSPHOLIPID FATTY ACID PATTERNS
 (% distribution)*

	16:0	16:1	18:0	18:1	18:2	20:3 ω 9	20:3 ω 6	20:4	18:1/ 18:2	20:3 ω 9/ 20:4
<i>Normal</i>										
0-1 month ^b (3)	26.0 ±1.5	3.1 ±0.6	20.8 ±1.0	20.6 ±0.3	2.2 ±0.7	0	4.5 ±0.6	21.7 ±0.9	12.2 ±4.4	0
1 month-1 year ^b (3)	23.2 ±0.6	2.0 ±0.2	22.3 ±1.1	20.8 ±0.6	3.7 ±1.5	0.5 ±0.5	5.0 ±0.8	22.6 ±1.9	7.2 ±2.1	0.020 ±0.020
1-5 years (4)	25.1 ±0.4	2.1 ±0.1	22.5 ±0.7	22.2 ±1.6	3.7 ±0.3	0	3.1 ±0.5	21.6 ±2.0	6.1 ±0.7	0
5-10 years (6)	26.7 ±1.9	2.6 ±0.7	21.3 ±0.7	21.6 ±1.6	4.3 ±0.2	0	2.4 ±0.6	21.4 ±3.0	5.2 ±0.5	0
Correlation coefficient ^c (16)	0.3858			0.3812		0.4219		-0.2949		0.4230
<i>p</i>	NS			NS		NS		NS		NS
<i>Lesion</i>										
1 month- 1 year ^d (3)	25.8 ±3.4	1.8 ±0.2	24.5 ±1.6	22.7 ±1.4	2.4 ±0.2	0	2.3 ±0.6	20.7 ±4.5	9.5 ±0.7	0
1-5 years (1)	25.3	3.9	19.8	20.6	4.6	0	1.2	24.7	4.5	0
5-10 years (4)	27.8 ±2.2	1.9 ±0.2	21.6 ±0.6	21.0 ±1.3	5.4 ±0.4	0	3.1 ±0.9	19.3 ±2.3	3.9 ±0.1	0
Correlation coefficient ^c (8)	0.2398			-0.2745		0.9491		-0.1637		-0.9109
<i>p</i>	NS			NS		<.001		NS		<.01

* 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 VII
 MEDIAL PHOSPHOLIPID FATTY ACID PATTERNS
 (% distribution)^a

	16:0	16:1	18:0	18:1	18:2	20:3 ω 9	20:3 ω 6	20:4	18:1/ 18:2	20:3 ω 9/ 20:4
<i>Normal</i>										
0-1 month ^b (3)	26.6 ±2.1	2.3 ±0.2	22.0 ±1.2	22.0 ±0.6	2.1 ±0.2	0	2.8 ±0.3	22.4 ±3.2	10.7 ±1.3	0
1 month- 1 year ^b (3)	25.3 ±0.8	2.3 ±0.3	20.8 ±0.7	24.6 ±0.9	3.7 ±1.4	0.4 ±0.4	2.6 ±0.2	20.4 ±1.4	8.4 ±2.5	0.019 ±0.019
1-5 years (4)	23.9 ±0.9	1.7 ±0.2	23.7 ±1.9	23.9 ±1.3	4.1 ±0.4	0.3 ±0.3	2.6 ±0.6	20.8 ±2.4	6.0 ±1.8	0.012 ±0.012
5-10 years (6)	22.7 ±1.8	1.9 ±0.4	22.7 ±1.2	21.8 ±1.4	4.1 ±0.6	0	2.7 0.6	22.0 ±3.6	6.0 ±1.0	0
Correlation coefficient ^c (16)	-0.5080			-0.4277	0.3491			-0.0610	-0.4631	
<i>p</i>	<.05			NS	NS			NS	NS	
<i>Lesion</i>										
1 month- 1 year ^d (3)	21.7 ±0.5	1.6 ±0.3	22.6 ±1.2	25.7 ±1.7	3.3 ±0.4	0.5 ±0.5	2.7 ±0.1	22.1 ±0.8	8.3 ±0.6	0.020 ±0.020
1-5 years (1)	31.3	8.1	18.2	21.8	5.3	0	1.5	13.9	4.1	0
5-10 years (4)	25.5 ±2.1	2.4 ±0.8	21.7 ±0.9	24.8 ±3.9	3.7 ±0.4	0	2.9 ±0.9	18.8 ±4.9	7.2 ±1.8	0
Correlation coefficient ^c (8)	0.4259			0.1120	0.0973			-0.2879	0.1338	
<i>p</i>	NS			NS	NS			NS	NS	

^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 VIII
SERUM PHOSPHOLIPID 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	>20:4	18:1/ 18:2	20:3ω9/ 20:4
Umbilical (10)	0.3 ±0.1	29.0 ±0.8	2.0 ±0.2	16.7 ±0.6	14.3 ±0.9	6.6 ±0.5	0.3 ±0.1	0 ±0	1.5 ±0.3	5.1 ±0.3	20.1 ±1.1	4.8 ±0.7	2.3 ±0.2	0.083 ±0.018
1 day-1 year (10)	0.1 ±0.1	33.9 ±2.0	3.1 ±0.2	15.9 ±0.6	23.3 ±1.8	8.2 ±1.1	0.8 ±0.1	0.1 ±0.1	1.1 ±0.4	3.1 ±0.3	8.7 ±1.4	1.7 ±0.4	3.3 ±0.4	0.186 ±0.079
1-5 years (17)	0.2 ±0.1	32.3 ±0.7	2.5 ±0.2	15.8 ±0.5	19.5 ±0.8	13.6 ±0.9	0.7 ±0.1	0.2 ±0.1	0.4 ±0.1	3.6 ±0.2	10.5 ±0.8	1.6 ±0.5	1.6 ±0.2	0.042 ±0.012
5-15 years (9)	0.3 ±0.1	30.8 ±0.9	2.0 ±0.3	16.5 ±0.7	18.6 ±1.8	18.6 ±1.2	0.5 ±0.0	0.3 ±0.1	0.3 ±0.1	4.0 ±0.6	9.0 ±0.8	0.6 ±0.3	0.9 ±0.1	0.029 ±0.010
15-30 years (9)	0.4 ±0.1	30.7 ±0.7	1.7 ±0.2	16.5 ±0.7	17.8 ±1.0	19.8 ±0.9	0.6 ±0.1	0.5 ±0.1	0.2 ±0.1	3.7 ±0.3	9.1 ±0.6	0.5 ±0.3	0.9 ±0.1	0.023 ±0.018
Correlation coefficient ^b (55)		-0.0796			-0.0977	0.6817						-0.2993	-0.2541	-0.5038
p		NS			NS	<.001						<.05	<.001	NS

^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.

rapidly than either free cholesterol or phospholipid. It is of interest to note that the amount of cholesterol ester present at age 10 in our studies is similar to that reported by Smith (1965) for this age, which is the lower limit of her study. It is also of interest to compare our data for normal intima with figures reported by other workers for whole arteries in children. Tuna and Mangold (1963) presented evidence that cholesterol ester is absent from the aortas of newborn infants. Scott *et al.* (1966) have also shown that cholesterol ester is present in coronary arteries in children in only negligible amounts. Meyer *et al.* (1966), however, found rather higher amounts of cholesterol ester in whole arteries from children, amounts roughly equal to the amount of free cholesterol present in their material.

The concentration of cholesterol ester in the lesion is greater than that in the normal intima, as might be expected, and the lesion concentration also shows an increase with age. These age changes in the intima, both normal and lesion, are in contrast to the rather constant concentration of cholesterol ester in both the normal media and in the media underlying the lesion.

Because the low amount of cholesterol ester in the intima of the younger age groups was assayed by gas-liquid chromatography, a means of studying the fatty acid composition of the earliest cholesterol esters found in the normal intima and in the lesions that arise in this intima was available. The cholesterol ester fatty acid pattern of the normal intima for the older group (5-10 years) was similar to that reported by Böttcher *et al.* (1960) for normal aorta in adults. However, the

findings of Böttcher *et al.* relate to the whole aorta rather than to the intima. Our figures for normal intima in this age group are somewhat lower with respect to linoleic acid and somewhat higher with respect to oleic acid than those reported for normal intima in adults by Smith (1965). However, our findings agree with those of Smith (1965) in that the fatty acid pattern of the intimal cholesterol esters resembles that of the cholesterol esters of the serum. The fact that the serum cholesterol ester fatty acid pattern alters during the first year of life, an observation reported previously by others (Renkonen, 1966; Zollner *et al.* 1966; Zee 1968), provides a very convenient tool for investigating the effect on normal intima of a changing serum cholesterol ester fatty acid pattern. Cholesterol ester appears in the normal intima during this time on an almost blank background. The cholesterol ester fatty acid pattern of the normal intima alters progressively over the first decade, increasing its linoleic and arachidonic acid content and decreasing its palmitic acid content. Apart from arachidonic acid, these changes follow, essentially, serum changes and provide circumstantial evidence that cholesterol ester accumulation in the normal intima during the first decade of life occurs as a result of infiltration from the serum. It is possible, of course, that the correspondence of serum and intima may be accounted for by contamination of the intimal surface with serum. We were aware, however, that the amount of cholesterol ester being handled in these specimens was extremely small and a good deal of care was taken in the washing and handling procedures to avoid possible contamination. The fact that the phospholipid fatty acid of the intima and lesion differs from the serum would make serum contamination with respect to cholesterol ester less likely. However, the amount of phospholipid present was rather higher than that of cholesterol ester so that the possibility of contamination accounting, in part, for the similarity of serum and intimal cholesterol ester remains.

With regard to atherosclerotic lesions, Böttcher *et al.* (1960) reported that atherosclerotic arteries contained cholesterol esters similar in composition to the serum. However, in the later work of Swell *et al.* (1960) and Nelson *et al.* (1961) in which the intima, media, and lesion were examined separately, these observations were disputed and it seems now well established that the cholesterol ester fatty acid composition of the fatty streak lesion (Geer and Guidry, 1964) and that of the "fat filled cells" (Smith, 1965; Smith *et al.* 1967) differs from that of the serum in having a higher proportion of oleic acid and a lower proportion of linoleic acid. In the children's lesions studied in the present series, the fatty acid composition of the cholesterol esters, however, resembles quite closely that of the surrounding normal intima and the lesions demonstrate the same shift of cholesterol linoleate with age. It is apparent, therefore, that although lesions below 1 year possess a high cholesterol oleate and low cholesterol linoleate content, that in these respects they resemble both the adjacent normal intima and also the serum to which they were exposed. The amount of eicosatrienoic acid in both normal intima and in lesion is lower than that reported for older individuals by others (Geer and Guidry, 1964; Geer and Malcolm, 1965; Smith, 1965, and Smith *et al.* 1967).

Since the lesion, as well as the normal intima, in the first decade of life contains cholesterol esters similar to those of the plasma, it seems probable that they arise primarily from the plasma rather than from local synthesis. However, in the lesions

processed for histology, lipid was found both intra- and extracellularly, so that the two processes may be involved. Firstly, serum cholesterol ester may infiltrate into the very early lesion, as appears to be the case for the cholesterol ester which accumulates in the normal intima, and subsequently, with the development of foam cells, more active processes play a role. The latter eventuality is certainly suggested by the analytical studies of adult fatty streak lesions carried out by Smith *et al.* (1967) and by the metabolic studies carried out in our own laboratory (Wahlqvist *et al.* 1969).

The normal intimal phospholipid fatty acid patterns are similar to those reported by others (Böttcher *et al.* 1960; Böttcher and Van Gent, 1961) for whole adult normal aorta except for the rather high arachidonic acid observed in all of the specimens examined in our children's series. What is perhaps of more interest is that there is no shift in the intimal phospholipid fatty acid pattern with age. In this respect and also, as far as the fatty acid patterns themselves are concerned, intimal phospholipid differs from serum phospholipid.

The phospholipid fatty acid pattern observed by Swell *et al.* (1960) and by Böttcher *et al.* (1961) for atherosclerotic plaques in adults is similar to that reported for the infant fatty streak lesions in the present paper. However, the proportion of phospholipid arachidonic acid in the infant lesions is rather higher than that reported by the workers referred to above for the adult lesion but similar to that for whole children's arteries reported by Wiese *et al.* (1967). For the youngest age groups studied, the phospholipid fatty acid patterns of normal intima and lesion differ from the pattern in the serum. While the pattern in the serum changes, that in normal intima and lesion does not. This is consistent with the view that the phospholipid which accumulates in atherosclerotic lesions has its origin in local synthesis (Zilversmit, 1959).

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