Relation of Age and Race to Serum Cholesterol Ester Fatty Acid Composition.*† (26033)

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The authors have shown(1) that there is a correlation between level of arachidonic acid in the serum cholesterol ester fatty acid (CEFA) fraction and species susceptibility to atherosclerosis. Those species (rat and dog) with a high level of arachidonic acid in their serum cholesterol esters are known to be resistant to production of atherosclerosis, while the more susceptible species (man, chicken, goose, rabbit and guinea pig) have low levels of that acid. Atherosclerosis starts to develop in man at a very early age(2) and does not reach its maximum severity until the fifth decade and beyond. It is claimed that during this period there is an increase in blood cholesterol level which may be related to development of the disease(3). Another factor may be the diet, which appears to bear a closer relationship to development of atherosclerosis than race(4). To provide more specific information regarding the suggested correlation of CEFA spectrum and susceptibility to atherosclerosis a study was carried out regarding relation of age and race to serum CEFA composition.

Methods and materials. All subjects reported in this study were males. The older subjects (negro and white), aged 60-87, were from the hospital medical wards. They were all in good nutritional status. The younger subjects (negro and white), aged 6-10 years old, were from an institution (D.C. Junior Village). Both age groups consumed the usual institutional diet containing approximately 30-35% fat. Fasting blood samples were obtained from the different subjects. Lipid extracts of the serum were prepared as previously described(5). Serum cholesterol levels were determined by the method of Sperry and Webb(6). Cholesterol esters were separated from the other lipid components by silicic acid chromatography(7). The isolated cholesterol esters were interesterified in HCl-methanol and methyl esters were sublimed according to the procedure of Stoffel *et al.*(8). Gas-liquid chromatography was carried out as previously described(9).

Results. The CEFA spectrum of the different groups is shown in Table I. In all subjects, regardless of age or race, the major fatty acid in the CEFA fraction of serum was linoleic acid (43.0-52.8%). Other fatty acids also occurred in substantial amounts: oleic (20.8-25.1%), palmitic (13.3-15.0%) and arachidonic acid (5.3-7.1%). Comparison of the different age groups, both negro and white, indicates that there were highly significant differences between the proportions of 2 of the fatty acids (oleic and linoleic) in serum cholesterol esters. Both negro and white children had significantly less oleic acid ($P<.01$) and significantly more linoleic acid ($P<.01$) than the older subjects. While older subjects had apparently higher percentages of arachidonic acid in their serum cholesterol esters, these differences were not considered significant since only values of $P<.01$ were considered significant in the present study. There were no significant differences between the groups in any of the other CEFA. Also, there were no significant differences in % oleic and linoleic acids of the CEFA between negro and white subjects of the same age group.

Table II shows blood cholesterol levels of the different subjects. Both negro and white children had significantly lower total blood cholesterols ($P<.01$) than older subjects. There were no significant differences in blood cholesterol levels of the 2 races.

Discussion. Our results clearly show that serum cholesterol esters of children have a significantly larger proportion of linoleic acid and less oleic acid than older individuals. At

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TABLE I. Relation of Age and Race to Serum Cholesterol Ester Fatty Acid Composition.

Fatty acid*		Negro†		White†	
Chain length carbons	No. double bonds	Avg age 7.7 yr (9)‡	Avg age 67 yr (13)	Avg age 8.5 yr (10)	Avg age 72 yr (13)
		% total fatty acids			
6 to 12		.5 ± .1§	1.2 ± .3	.5 ± .1	1.0 ± .2
14	0	.9 ± .2	1.0 ± .3	.9 ± .2	.9 ± .1
14	1	.2 ± .1	.4 ± .2	.2 ± .0	.4 ± .1
16	0	13.7 ± .9	15.0 ± 1.6	13.3 ± 1.1	13.8 ± 1.5
16	1	3.0 ± 1.0	4.2 ± 1.1	3.2 ± .4	4.4 ± 1.5
16	2	.3 ± .1	.3 ± .1	.4 ± .0	.4 ± .1
18	0	1.5 ± .5	1.3 ± .5	1.2 ± .4	1.5 ± .5
18	1	20.9 ± 1.2	25.1 ± 2.7	20.8 ± 1.4	24.9 ± 2.7
18	2	51.8 ± 2.5	43.0 ± 3.5	52.8 ± 1.8	44.7 ± 4.0
18	3	.5 ± .1	.5 ± .4	.5 ± .1	.6 ± .3
20	0	.4 ± .1	.5 ± .3	.6 ± .2	.5 ± .2
20	3	.3 ± .1	.4 ± .1	.3 ± .0	.4 ± .3
20	4	6.0 ± .5	7.1 ± 1.7	5.3 ± .9	6.5 ± 1.7

* Represents major fatty acids determined. Small amounts of others were also detected.
† All subjects were males.
‡ Figures in parentheses indicate No. of subjects.
§ Stand. dev.

TABLE II. Influence of Age and Race on Serum Cholesterol Level.

Avg age, yr*	Race	Cholesterol, mg % Free	Ester	Total	Ester, % Total
7.7	Negro	40 ± 4	138 ± 12	178 ± 16	77.5 ± 1.3
8.5	White	40 ± 9	144 ± 28	184 ± 35	78.3 ± 3.3
67	Negro	63 ± 8	166 ± 26	229 ± 32	72.5 ± 2.3
72	White	61 ± 15	162 ± 35	223 ± 50	72.7 ± 1.4

* No. of subjects indicated in Table I.

the same time, younger individuals also have a lower blood cholesterol level. It might be suggested that level of linoleic acid in the serum CEFA fraction bears an inverse relationship to serum cholesterol. Of particular interest is the finding that there is very little change in % arachidonic acid in the serum CEFA with advancing age. The level of that acid in the serum CEFA fraction appears to be a characteristic of the species(1) and does not appear to be affected by age. This may also be related to the observation that man is susceptible to atherosclerosis and that the disease starts to develop at an early age. The question still to be answered is whether the arachidonic acid level of serum CEFA in man can be altered by experimental means.

Summary. The serum CEFA and cholesterol level of children (6-10 years old) and older subjects (60-87 years old) of both negro and white races have been compared. Children of both races had significantly less oleic acid and significantly more linoleic acid in their serum CEFA fraction than older subjects. Arachidonic acid did not show significant changes with increasing age. Negro and white subjects of the same age group did not show significant differences in CEFA spectrum. Also, children had a significantly lower total blood cholesterol level than older individuals. The significance of these findings as related to atherosclerosis is discussed.

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Identification of An Antigen Common to *Listeria monocytogenes* and Other Bacteria.* (26034)

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An antigen common to Gram-positive bacteria was described by Rantz and associates (1,2) and referred to as non-species specific antigen. Subsequent studies revealed that this heterogenetic antigen is present in many but not all species of these bacteria. For example, it was not detected in *Staphylococcus citreus* and *Micrococcus lysodeikticus*, even after lysis of the latter microorganisms by lysozyme(3,4). The antigen is produced by certain but not all species of the genus *Bacillus* (5). The present study was undertaken to determine whether *Listeria monocytogenes* produces this antigen and, also, whether it is this antigen that is the basis for the serologic crossreactions between this microorganism and *Staphylococcus* described by Seeliger(6) and Welshimer(7).

Materials and methods. Strains of *L. monocytogenes* (types 1, 2, 3, 4a, 4b) were received through the kindness of Dr. J. T. Barrett, Univ. of Missouri, and Dr. M. L. Gray, Montana State College. The former strains were obtained from CDC (KC 222 to 226). Dr. Gray's strains types 1 and 4a originated from the National Type Culture Collection (#5349 and #5214); types 2 and 4b from Dr. Donker-Voet, Utrecht, Netherlands; type 3 was isolated from a fatal case of meningitis in Muskegon, Mich. Dr. H. J. Welshimer made available one strain each of *L. monocytogenes* type 1 and *Staphylococcus epidermidis*. Strains of *S. aureus* and *Bacillus subtilis* were isolated in this laboratory, *S. aureus* strain D was obtained from Difco Laboratories, Detroit. The strains were maintained on blood agar.

For preparation of antigens, the microorganisms were grown on brain veal agar in Kolle flasks at 37°C for 18 hours. To each flask was added 20 ml of phosphate buffer of pH 7.3. The suspensions were centrifuged immediately at 23,500 G for 20 min in a refrigerated centrifuge. Supernates of infusion broth cultures were also used. Supernates, notably of type 4a, proved to be hemolytic; it was observed that heating at 56°C for 15 min abolished this effect. Therefore, the antigens were routinely heated and then kept at 4°C until used.

The hemagglutination test was carried out as previously described(3,8). Briefly, human red blood cells (blood group O) were washed 3 times, mixed with antigen in suitable dilutions, and incubated for 30 min at 37°C. The modified erythrocytes were washed 3 times and then used in the hemagglutination test. Antiserum in serial 2-fold dilutions (vol. 0.2 ml) was mixed with 0.2 ml of the modified red blood cell suspension. The mixtures were incubated in a waterbath at 37°C for 30 min, and the resulting hemagglutination was read grossly after centrifugation at 1300 G for 3 min. *L. monocytogenes* antisera were obtained from Dr. Gray. Others were prepared in this laboratory by injection, 2 days apart, of suspensions of agar grown bacteria (2 ml) that had been heated at 56°C for 15 min, followed one week later by injection of unheated suspensions. The animals were bled one week after immunization.

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