Lipid Fatty Acid Composition of Several Areas of the Aorta in Subjects with Atherosclerosis.* (26209)

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It has been suggested(1) that essential fatty acids are required for normal transport and metabolism of cholesterol. According to that hypothesis, when there is a deficiency of those acids cholesterol is esterified with increased amounts of saturated and monoenoic acids and these esters accumulate in the tissues (aorta) producing atherosclerotic plaques. This suggested relationship has prompted a number of studies(2-5) to ascertain if there are any differences in the fatty acid composition of the cholesterol ester fraction of serum and atheromatous plaques. The published data(2-5) are at variance with one another and indicate that the percentage of polyunsaturated fatty acids in the cholesterol ester fatty acids (CEFA) of atheroma may be lower, higher or the same as that fraction in serum. Some of the differences in the earlier data may be due to the fact that the analyses of the cholesterol ester fraction of the aorta were carried out either on wall tissue (intima plus media), plaques plus wall tissue or plaques only. The media accounts for the largest portion of the aorta and the cholesterol ester fraction of that area could dilute out any apparent differences in CEFA composition attributable to the atherosclerotic lesion of the intima. To circumvent some of these difficulties, we recently reported a study(6) in which the CEFA composition of the media (freed from intima), plaque material and serum were compared. It was shown that the cholesterol ester fraction of plaque material had significantly less linoleic acid and more oleic acid than either media or serum. The purpose of the present study was to extend those earlier observations to another area of the aorta (thickened intima) and to obtain data on the triglyceride and phospholipid fractions of several areas of the aorta and serum.

Methods and materials. The tissues, other than serum, were obtained at autopsy from 6 hospitalized male patients, aged 38, 39, 51, 63, 68, and 78 years.† Autopsies were performed within 8 to 12 hours after death and portions of the abdominal aorta removed. The aorta was freed of adherent tissue and its layers carefully dissected along their cleavage planes into adventitia, media and intima. The intima was partitioned, as far as their occurrence permitted, into normal appearing portions, thickened portions or early plaques, and free-lying lipid containing material from far-advanced plaques. Except for one subject, normal appearing intima was not available in sufficient quantities for analysis. Fasting serum was obtained from 9 male patients aged 55-70 years, with occlusive atherosclerosis in a good nutritional status. The tissues were quick frozen in a dry ice acetone mixture, pulverized and initial lipid extractions carried out with 1:1 acetone-alcohol(7) followed by 2:1 chloroform-methanol. Serum was extracted in a similar manner. Cholesterol esters, triglycerides and phospholipids were separated by chromatography on silicic acid(8), with the modification that 100% methanol was used to elute the phospholipid fraction. The minor lipid components were eluted with 25% ethyl-ether in petroleum ether and discarded before elution of the phospholipid. The isolated lipid fractions were interesterified in HCl methanol and methyl esters sublimed according to the method of Stoffel *et al.*(9). Gas-liquid chromatography was carried out as described earlier(6) with a succinate polyester of diethylene glycol as the stationary phase.

Results. Cholesterol ester fatty acid composition (Table I). As in our earlier report (6), media and serum had approximately the

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† The patients suffered from a variety of diseases and had varying degrees of atherosclerosis.

TABLE I. Cholesterol Ester Fatty Acid Composition of Several Areas of Aorta and Serum.

Fatty acid*		Area of aorta†			
Chain length carbons	No. double bonds	Media	Intima thickened	Plaque material	Serum‡
		% total fatty acids			
6 to 12		.6 ± .1§	1.1 ± .8	1.2 ± .6	.9 ± .2
14	0	1.5 ± .3	1.5 ± .8	1.0 ± .3	.7 ± .4
"	1	.4 ± .1	.4 ± .2	.4 ± .2	tr
16	0	15.8 ± .6	17.5 ± 3.6	17.7 ± 1.4	15.9 ± 1.9
"	1	5.5 ± 1.6	6.0 ± 2.0	5.6 ± 1.1	4.6 ± 1.0
"	2	.6 ± .2	.7 ± .4	.5 ± .2	.5 ± .2
18	0	1.7 ± 1.2	2.0 ± .9	1.6 ± .5	2.9 ± .7
"	1	27.6 ± 2.7	36.7 ± 4.3	38.4 ± 3.0	26.2 ± 1.1
"	2	37.9 ± 3.2	28.8 ± 6.4	28.1 ± 1.8	40.8 ± 2.4
"	3	.4 ± .1	.4 ± .1	.6 ± .2	.4 ± .1
20	0	.4 ± .1	.4 ± .1	.5 ± .1	.4 ± .1
"	3	.4 ± .1	.6 ± .5	.7 ± .5	.4 ± .1
"	4	7.2 ± 1.1	3.9 ± 1.7	3.7 ± .9	6.3 ± 1.0
Saturated		20.0	22.5	22.0	20.8
Mono-unsaturated		33.5	43.1	44.4	30.8
Polyunsaturated		46.5	34.4	33.6	48.4

* Represents major fatty acids determined; small amounts of others were also detected.
† Values represent avg of 6 subjects.
‡ Values represent avg of 9 subjects.
§ ± stand. dev.

same CEFA composition. The major acid of the CEFA in those tissues was linoleic acid which accounted for approximately 40% of total acids. Thickened intima (early plaques) and plaque material had almost the same CEFA composition. CEFA of both of those areas of the aorta (thickened intima and plaque material) were different from media and serum, in that they contained significantly less ($P<.01$) linoleic and arachidonic acids and more oleic acid. No significant differences were found between the minor fatty acids of the several tissues.

Triglyceride fatty acid composition (*Table II*). In the tissue triglyceride fraction, palmitic and oleic acids comprised from 72-

TABLE II. Triglyceride Fatty Acid Composition of Several Areas of the Aorta and Serum.*

Fatty acid		Area of aorta			
Chain length carbons	No. double bonds	Media	Intima thickened	Plaque material	Serum
		% total fatty acids			
6 to 12		1.1 ± .5	1.3 ± .7	2.1 ± 2.1	.8 ± .3
14	0	2.1 ± .8	1.9 ± .5	2.2 ± .7	1.4 ± 1.0
"	1	.5 ± .1	.6 ± .2	.5 ± .3	.2 ± .1
16	0	31.9 ± 2.7	30.9 ± 1.5	31.9 ± 3.0	30.0 ± 3.0
"	1	3.3 ± 1.5	3.4 ± .9	2.9 ± .8	3.8 ± .7
"	2	.4 ± .2	.6 ± .3	.6 ± .5	.3 ± .1
18	0	11.1 ± 4.5	8.2 ± 2.9	7.6 ± 1.5	6.6 ± 1.6
"	1	40.2 ± 5.6	42.1 ± 4.3	40.9 ± 3.6	43.8 ± 2.6
"	2	8.2 ± 1.9	9.8 ± 2.3	10.4 ± 2.0	11.6 ± 2.2
"	3	.4 ± .1	.4 ± .2	.2 ± .1	.4 ± .3
20	0	tr	tr	tr	.3 ± .2
"	4	.8 ± .5	.8 ± .3	.7 ± .2	.8 ± .4
Saturated		46.2	42.3	43.8	39.1
Mono-unsaturated		44.0	46.1	44.3	47.8
Polyunsaturated		9.8	11.6	11.9	13.1

* See footnotes to Table I.

TABLE III. Phospholipid Fatty Acid Composition of Several Areas of the Aorta and Serum.*

Fatty acid: Chain length carbons	Fatty acid: No. double bonds	Area of aorta: Media	Area of aorta: Intima thickened	Area of aorta: Plaque material	Serum
		% total fatty acids			
6 to 12		.8 ± .4	1.0 ± .4	1.3 ± .5	.6 ± .2
14	0	2.1 ± 1.1	1.9 ± 1.1	2.5 ± 2.0	.8 ± .4
"	1	.7 ± .2	.4 ± .2	.7 ± .6	.4 ± .2
16	0	35.9 ± 1.5	36.2 ± 2.8	31.3 ± 6.2	39.2 ± 3.5
"	1	2.2 ± 1.0	2.9 ± .9	4.2 ± 2.1	2.2 ± .3
18	0	19.8 ± 2.5	16.4 ± 2.3	15.3 ± 4.6	12.6 ± 2.9
"	1	18.9 ± 2.4	22.1 ± 1.5	22.9 ± 3.8	24.5 ± 2.5
"	2	5.6 ± .7	7.4 ± 3.5	8.7 ± 4.1	11.4 ± 2.0
"	3	.5 ± .2	.4 ± .1	.2 ± .2	tr
20	0	.4 ± .2	.3 ± .1	tr	.4 ± .1
"	3	.2 ± .2	.3 ± .1	tr	.5 ± .2
"	4	10.2 ± 2.3	2.6 ± 1.0	1.8 ± 1.1	2.4 ± .5
22	→	2.7 ± 1.7	8.1 ± 2.5	11.1 ± 2.6	5.0 ± 2.5
Saturated		59.0	55.8	50.4	53.6
Mono-unsaturated		21.8	25.4	27.8	27.1
Polyunsaturated†		19.2	18.8	21.8	19.3

* See footnotes to Table I.
† Includes long chain fatty acid fraction, 22 carbons and beyond.

75% of the total fatty acids. Percentages of palmitic and oleic acid were strikingly constant and were in the narrow range of approximately 29-35% and 39-46% respectively. There were only small differences in the remaining triglyceride fatty acids of the several tissues. The most notable was the high level of stearic acid in the media (11.1%). Comparison of CEFA and triglyceride fatty acids indicates that there was a much lower level of polyunsaturated fatty acids in the triglyceride fraction.

Phospholipid fatty acid composition (Table III). The phospholipid fraction of the tissues examined was characterized by a high level of saturated fatty acids. This fraction accounted for 50-59% of total fatty acids. The major fatty acids of the phospholipid fraction were palmitic, stearic and oleic acids. The phospholipid fraction of the media had significantly ($P<.01$) more arachidonic acid than that fraction in the other tissues. The phospholipid fraction of the thickened intima and plaque material were similar in fatty acid composition, but contained a greater proportion of long chain fatty acids than either media or serum.

Discussion. Our present results have confirmed our earlier observations(6) and have provided new data regarding fatty acid distribution in the lipid fractions of several different areas of the human aorta. A direct comparison of the data obtained in this study cannot be made with that of earlier reports(2-5) since in the present experiments the aortic media was separated and analyzed independently of the plaques (early and advanced). Of the different lipid fractions analyzed in the several areas of the aorta, the cholesterol ester fraction showed the greatest differences in fatty acid distribution. Distinct differences were noted in CEFA composition of media, serum and plaques (early and advanced). This dissimilarity in CEFA composition of the plaques and serum indicates that there may be a distinct mechanism operating in which cholesterol esters of a more saturated and monoenoic nature are laid down as atherosclerosis progresses (1). It is also of interest that both the early and advanced plaques had approximately the same CEFA composition. The diversity in CEFA composition of aortic media and plaques is particularly noteworthy and provides suggestive evidence that there are distinct differences in the metabolism of the cholesterol ester fraction in those areas of the aorta. The fatty acid distribution in the

triglyceride fraction of the several areas of the aorta and serum were similar. It has been observed that there is an increase in the triglyceride and phospholipid content of the intima as atherosclerosis develops, as well as in free and ester cholesterol(10). This, plus the similarity of the triglyceride fatty acid composition in media, serum and plaques suggests that there is an indiscriminate deposition of triglyceride in the aorta. While there were some differences in fatty acid distribution of the phospholipid fraction in the several areas of the aorta and serum, it should be pointed out that that fraction was heterogeneous since it contained lecithins, sphingomyelins and cephalins. The differences in the percentages of the polyunsaturated fatty acids in that fraction of the several tissues studied might be due to the greater abundance of a specific phospholipid. The results of the present study stress the need for further study of the origin and metabolism of the cholesterol ester fraction in the aorta.

Summary. The cholesterol ester, triglyceride and phospholipid fatty acid composition (gas-liquid chromatography) of aortic media, thickened intima and plaque material were determined in 6 human subjects. For comparison, the fatty acid composition of those fractions in serum was determined in 6 subjects (aged 55-70 years) with occlusive atherosclerosis in a good nutritional status. The triglyceride fraction of those tissues and serum were similar in their fatty acid composition. Some slight differences were noted in the fatty acid composition of the phospholipid fractions, most notably in the arachidonic and long chain fatty acids. The cholesterol ester fraction of the tissues studied showed the greatest differences in fatty acid composition. Both early and advanced plaques had significantly less linoleic and more oleic acid in that fraction than serum or media. Media and serum CEFA were similar in their fatty acid composition. The significance of these findings in relation to atherogenesis is discussed.

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Evaluation of Respiratory Mutants for Selecting Compounds for Cancer Chemotherapy Study.*† (26210)

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Many investigators, in their search for systems which might respond similarly to tumor tissue because of some parallelism of metabolism, have attempted to establish a preliminary screen for cancer chemotherapy utilizing organisms which might be induced to preferentially use fermentative metabolism. Studies with anaerobes by Bradner and Clarke(1) as well as by DiPaolo and Rosenfield(2) indicated that results with these sys-

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