Correlation of Arachidonic Acid of Serum Cholesterol Esters in Different Species with Susceptibility to Atherosclerosis.* (25823)

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Data on serum cholesterol ester fatty acid (CEFA) composition of different species are limited. Cow and human plasma cholesterol esters have been shown(1-6) to contain a large proportion of linoleic acid (40-50% of total CEFA), while arachidonic acid accounts for only 5-10%. The CEFA of dog plasma (7) contain a much higher proportion (up to 25%) of arachidonic acid than that of man, but in this species linoleic acid also predominates. Rat blood cholesterol esters have been reported(8) to consist principally of saturated fatty acids, while in another study(9) oleic acid was found to be the major CEFA.† Sinclair(10) has recently hypothesized that a deficiency of arachidonic acid, or the factors necessary for arachidonic acid synthesis (linoleic acid and pyridoxine), may be important in development of atherosclerosis in man. Recent studies(6,11) from these laboratories with the aid of gas-liquid chromatography have shown that arachidonic acid constitutes up to 50% of serum CEFA of the rat and only 7.5% in serum CEFA of man. The correlation between arachidonic acid of the CEFA of those 2 species and their well known differences in susceptibility to development of atherosclerosis suggested that determination of the CEFA of other species would be of interest.

Methods and materials. All animals used in this study were males, with one exception noted in Table I. The animals (5-6 per group) were as follows: rats (Wistar strain, 250-350 g), guinea pigs (Hartley strain, 300-350 g), chickens (White Leghorn, 1-2 years), geese (mixed breeds, 1-2 years), pigs (Yorkshire and Hampshire, 1-2 years), rabbits (albino, 6 months), dogs (mongrels, 1 year and older). The diets of the different species and their fatty acid composition are indicated in Table III. The animals were fasted overnight prior to collection of blood samples. Blood was obtained from 6 fasted human subjects, aged 61-74 years, in good nutrition, on the usual hospital diet (30-40% fat). Lipid extracts of individual serum samples and diets were prepared as described earlier(12). Free and total cholesterol of each extract were determined by the method of Sperry and Webb (13). Cholesterol esters were separated from the other lipid components by chromatography on silicic acid(14). The isolated cholesterol esters were interesterified in HCl-methanol and methyl esters were sublimed according to procedure of Stoffel *et al.*(15). Gas-liquid chromatography was carried out as previously described(6).

Results. Serum CEFA compositions of the different species are shown in Table I. The rat had the highest content (50% of arachidonic acid in the serum cholesterol ester fraction followed by the dog with 17%. The remaining species (man, chicken, goose, rabbit, pig and guinea pig) had much smaller amounts of arachidonic acid in the cholesterol ester fraction. Linoleic acid was the major acid in dog, man, rabbit, guinea pig and pig, with the guinea pig having the highest percentage (57.9%) of that acid. The goose and chicken had substantial amounts of linoleic acid (approximately 30%), but oleic acid was the major acid in the cholesterol esters of those species. Also, there were differences in distribution of saturated and other monoenoic acids in the cholesterol ester fractions characteristic of the different species.

Table II shows serum cholesterol levels of the different species. Man and goose had the highest average total cholesterol level

* This study supported by grants from PHS and Am. Heart Assn.

† While this paper was in preparation, Howton, D. R., and Hashimoto, S., *Am. J. Clin. Nutrition*, 1960, v8, 50, also reported high levels of arachidonic acid (67.6%) in rat plasma cholesterol esters.

TABLE I. Serum Cholesterol Ester Fatty Acid Composition of Different Species.

Fatty acid* Chain length carbons	No. double bonds	Rat (5)†	Dog (5)	Man (6)	Goose (6)	Chicken (6)	Rabbit (6)	Pig (5)	Guinea pig (6)
		% total fatty acids							
6 to 12		1.7 ± .4‡	.7 ± .3	1.0 ± .2	.8 ± .1	.4 ± .2	.7 ± .2	.8 ± .1	2.8 ± .5
14	0	.8 ± .2	.6 ± .4	.9 ± .2	.3 ± .1	.3 ± .1	.8 ± .2	.5 ± .1	1.1 ± .5
,,	1	.5 ± .3	.2 ± .1	.3 ± .1	tr	tr	.6 ± .2	.2 ± .0	.6 ± .4
16	0	12.4 ±2.1	12.2 ±1.9	13.7 ±1.4	17.2 ±1.5	15.1 ±2.2	21.3 ±2.0	12.8 ±2.0	18.6 ±3.4
,,	1	2.4 ±1.1	2.9 ±1.0	4.1 ±1.1	1.7 ± .6	6.9 ±1.5	2.1 ± .6	2.9 ± .7	2.4 ± .6
,,	2	tr	tr	.4 ± .1	tr	tr	tr	.2 ± .0	tr
18	0	2.6 ± .3	3.2 ±1.1	1.3 ± .3	2.4 ± .3	6.1 ±1.9	3.5 ±1.2	4.2 ± .9	.5 ± .2
,,	1	9.8 ± .9	18.1 ±1.6	25.8 ±3.5	40.0 ±8.1	35.8 ±8.9	24.6 ±3.4	30.8 ±3.7	14.8 ±2.5
,,	2	19.5 ±1.9	44.9 ±6.3	43.3 ±4.7	31.3 ±7.7	29.3 ±7.1	43.0 ±3.2	45.4 ±2.5	57.9 ±3.1
,,	3	.3 ± .3	tr	.7 ± .4	.3 ± .2	.3 ± .1	1.1 ± .9	.4 ± .2	.2 ± .2
20	0	tr	tr	.7 ± .4	.2 ± .1	.2 ± .1	tr	tr	tr
,,	3	tr	.2 ± .1	.3 ± .2	tr	.4 ± .2	tr	tr	tr
,,	4	50.0 ± .9	17.0 ±6.5	7.5 ±2.2	5.8 ± .3	5.2 ±2.1	2.3 ± .5	1.8 ± .8	1.1 ± .7

* Represents major fatty acids found; small amounts of others were detected. † Values in parentheses represent No. of animals; animals were males with exception of 3 geese. ‡ Stand. dev.

(227 and 228 mg respectively). The dog had an intermediate total serum cholesterol level of approximately 130 mg% and the remaining species had levels less than 100 mg%. In all species studied, percent of esterified cholesterol in total serum cholesterol was in the narrow range of from 69.2 to 79.0%.

Diets of the different species (Table III), except man, were low in fat (2.4-4.2%). Appreciable amounts of linoleic acid (29.3-54% of total dietary fatty acids) were present in fat of all diets. The diets of the rat, rabbit and guinea pig contained approximately 1% of linoleic acid. That of the chicken and goose contained 1.7%. The remainder of the dietary fatty acids were principally palmitic and oleic acids.

Discussion. The serum CEFA pattern is different for each species and does not appear to be related to dietary intake of fatty acids. Those species (goose and chicken) which consumed a diet high in linoleic acid had low percentages of linoleic and arachidonic acids in their serum cholesterol ester fractions, whereas the CEFA of the rat, which consumed a diet containing less linoleic acid, had the greatest amount of polyunsaturated fatty acids. It would appear that those animals with high CEFA arachidonic acid levels (rat and dog) can more easily utilize their linoleic acid for arachidonic acid production or may incorporate more of the available arachidonic acid into cholesterol esters.

Data in Table I indicate that there is a correlation between susceptibility to atherosclerosis of the different species and content of arachidonic acid in cholesterol esters of their sera. The rat is extremely resistant to production of atherosclerosis by artificial means(16,17). The dog is also highly resistant by the usual cholesterol feeding technic, although the disease can be produced if thiouracil is added to a high cholesterol diet(18). In the dog arachidonic acid in CEFA, while high, is considerably lower than in the cholesterol ester fraction of the rat. In the goose, chicken, rabbit, guinea pig and pig atherosclerosis has been readily produced by feeding of cholesterol(19-23). In those species the atheromatous lesion bears a marked simi-

TABLE II. Serum Cholesterol Levels of Different Species.

Species*	Serum cholesterol, mg % Free	Ester	Total	$\frac{\text{Ester}}{\text{Total}}$, %
Rat	20.6 ± 1.8†	41.5 ± 13.5	70.1 ± 13.0	69.9 ± 5.6
Dog	31.1 ± 8.9	98.3 ± 37.5	129.4 ± 45.3	76.0 ± 4.7
Man	60.0 ± 9.9	166.5 ± 36.9	226.5 ± 46.5	73.5 ± 1.6
Goose	70.4 ±20.2	158.0 ± 30.9	228.4 ± 38.0	69.2 ± 7.4
Chicken	33.7 ± 9.8	119.2 ± 35.9	152.9 ± 45.2	78.0 ± 2.0
Rabbit	11.8 ± 3.3	27.4 ± 10.3	39.2 ± 12.6	69.9 ± 5.5
Guinea pig	10.8 ± 2.3	32.3 ± 7.7	43.1 ± 9.5	74.9 ± 3.2
Pig	14.7 ± 3.6	55.4 ± 9.9	70.1 ± 12.4	79.0 ± 3.3

* Represents avg of 5 to 6 animals. † Stand. dev.

larity to that of man. All of those species were found to have low arachidonic acid levels in their serum cholesterol esters(1.1-5.8%). Man is susceptible to atherosclerosis, but seems to be somewhat more resistant than the other species (arachidonic acid 7.5%). The disease takes many years to progress to important lesions in man, while only a short high-cholesterol feeding period is necessary for its production in the susceptible species. It is also of interest that spontaneous atherosclerosis(24-27) has been observed in goose, chicken, rabbit and swine. The dog develops atherosclerosis spontaneously, but lipid appears to occur to a minimal extent or is absent from the lesion(28). No definite correlation between levels of fatty acids, other than arachidonic, in the CEFA and susceptibility to atherosclerosis is apparent. Also, there is no correlation between serum cholesterol level and species susceptibility to atherosclerosis.

TABLE III. Dietary Fatty Acid Composition and Fat Content.

Fatty acid: Chain length carbons	No. double bonds	Diets of species*: Rat	Dog	Chicken, goose	Rabbit, guinea pig
		% total fatty acids			
6 to 12		.3	.4	tr	.3
14	0	1.6	1.0	tr	.6
”	1	.4	tr	tr	tr
16	0	23.6	28.1	13.7	18.3
”	1	2.0	1.6	.2	.9
18	0	8.5	7.1	2.3	2.5
”	1	32.1	26.1	28.9	23.4
”	2	29.3	33.2	54.0	48.6
”	3	2.2	2.5	.9	5.4
Dietary fat content,† %		4.2	2.5	3.1	2.4
Linoleic acid content,‡ %		1.0	.7	1.7	1.1

* Represents analyses of Purina rat chow, Purina rabbit and guinea pig chow, cracked corn (chickens and geese), and Purina dog chow.

† Fat content of diets consumed by the pigs was unavailable.

‡ Calculated as % of diet.

Our results are in accord with Sinclair's hypothesis(10) that a deficiency of arachidonic acid may be an important factor in etiology of atherosclerosis. These findings are evidence only by association. It remains to be seen whether levels of arachidonic acid in the tissues, perhaps particularly in fatty acids of cholesterol esters, may be increased and whether that increase in arachidonic acid may reduce susceptibility to atherosclerosis. The low content of fat in the diets of species other than man suggests that differences in proportion of arachidonic acid in the CEFA may be a characteristic of the species which will not be readily affected. The large amounts of saturated, monoenoic and short-chain fatty acids and the uncertainty of the adequacy of pyridoxine content in the modern diet of "western" man leaves open the possibility of increasing arachidonic acid content of his tissues.

Summary. Serum CEFA composition of 8 different species has been determined by gas-liquid chromatography. Different CEFA spectrums were found for each species. The outstanding variation was the proportions of arachidonic acid in CEFA of the serums. That of the rat had 50% of arachidonic acid and that of the dog had 17%. All species also had a large proportion of linoleic acid in the CEFA of their serums. The rat and the dog, with high arachidonic acid levels in CEFA of their serums, are known to be highly resistant to development of atherosclerosis.

Those species having small proportions of arachidonic acid in the CEFA of their serums are susceptible to atherosclerosis and develop the disease spontaneously. This study provides evidence, by association, that level of arachidonic acid in the CEFA may be related to susceptibility to atherosclerosis. It remains open to investigation whether the proportion of arachidonic acid in the CEFA is a characteristic of the species or whether it, and the susceptibility to atherosclerosis, can be altered by dietary measures.

The authors gratefully acknowledge the technical assistance of Ernest Stutzman, David F. Boyd, Melvin D. Law, and Richard Towner.

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Received April 14, 1960. P.S.E.B.M., 1960, v104.

Association between Presence of Transplanted Leukemia and Morphological Changes in Hosts' Thyroid Glands.* (25824)

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We observed that presence of spontaneous mammary adenocarcinoma in C3H parous females is associated with decreased epithelial height and increased diameter of thyroid follicles as compared to non-tumor-bearing controls(1). These changes suggested that presence of tumor is associated with decreased thyroid function of host(2). This investigation was initiated to determine if this phenomenon might be more widespread in its occurrence, as suggested by earlier observations of Larinow(3) that mice with various types

* The work supported in part by grant to Indiana Univ. Med. Center from Am. Cancer Soc.

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