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Cholesterol-Esterifying Activity of Aortas from Atherosclerosis-Resistant and Atherosclerosis-Susceptible Species[1] (37754)

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Cholesterol esterification by arterial tissue has been demonstrated in rats (1), rabbits (2, 3), man (4), and pigeons (5). *In vivo* studies indicate that cholesterol esterification within the arterial wall contributes measurably to the cholesteryl ester content of atherosclerotic rabbit aortas (6). The magnitude of stimulation of cholesterol esterification with atherogenesis (2) suggests that this process may be responsible for the accumulation of the cholesteryl ester in the atheroma.

Susceptibility to atherosclerosis varies widely among different species, and within any species susceptibility varies from one anatomical site to another. The reason for these differences, which are for the most part unexplained, has sparked considerable interest; any metabolic or structural feature which displays a high correlation with susceptibility would *ipso facto* provide an important clue to critical mechanisms of atherogenesis. Resistance to atherogenesis has been attributed to a high ratio of alpha to beta lipoproteins in plasma (7); to high lipolytic activity in the arterial wall (8); to homeostatic mechanisms which resist the development of hypercholesterolemia (9); and inversely to the acid mucopolysaccharide content of the arterial wall (10). While probably all valid, these phenomena fail to explain many of the known species differences and anatomical differences in propensity to atherogenesis.

In the present investigation, the possibility was considered that the propensity of arterial tissue to develop atherosclerosis was related to the innate capacity of the tissue to esterify cholesterol. To test this hypothesis, we examined the cholesterol-esterifying activity in aortas derived from several species which are resistant and susceptible to experimental atherosclerosis, and compared activities of thoracic and abdominal aortic segments in some of these species.

Methods. The following species were used: male mongrel dogs (average wt 16.3 kg); male New Zealand rabbits (average wt 2.8 kg); male Wistar rats (average wt 270 g); cockerels (average wt 3.2 kg); male (average wt 526 g) and female (average wt 464 g) Show Racer pigeons; male (average wt 471 g) and female (average wt 470 g) White Carneau pigeons. Level of maturation was roughly comparable (young adults) for all species, except in the dogs whose ages were not accurately known. Animals were studied on conventional (nonatherogenic) diets, and the pigeons were studied while young (6 months) and free of atherosclerosis, since we were interested in determinants of atherogenesis rather than its consequences. Dogs and rabbits were killed with pentobarbital; the remaining animals were decapitated.

Thoracic and abdominal aortas were removed and stripped of periarterial fat. The aortas were cut along the longitudinal axis, and the adventitia was removed by scraping with a razor blade. Cell-free homogenate of the remaining tissue—intima + media + shreds of adventitia (approximately 1.8 g)—was prepared in 0.1 *M* phosphate buffer (7.4), and the microsomes were isolated as described previously (2).

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The composition of the incubation medium is given in the Tables. The reaction mixture was incubated at 37° for 4, 8, and 16 min under air. Incubation medium with substrate but without microsomes was used as a control. After incubation the reaction was stopped with 20 ml chloroform–methanol (2:1). Lipid was isolated from the chloroform–methanol extract by the procedure of Folch *et al.* (11). Cholesteryl ester was isolated by thin-layer chromatography of the lipid on silica gel G using 5% diethyl ether–97% petroleum ether (boiling range 60–90°). After development, the cholesteryl ester area was scraped into a counting vial, suspended in a thixotropic gel (12), and assayed for radioactivity in a Packard liquid-scintillation spectrometer. Protein concentration of the microsomal suspension was determined by the method of Lowry *et al.* (13).

Results. Esterification of cholesterol by aortic microsomes derived from several species was examined using [1-^{14}C]palmityl-CoA as a substrate. As shown in Table I, incorporation of radioactivity into cholesteryl ester increased progressively with time of incubation. Cholesterol-esterifying activity of microsomes from thoracic aortas varied with the species. Activity of microsomal material from rat thoracic aorta was 3.6 times as great as that from dogs and rabbits and 6.5 times as great as that from cockerels. Cholesterol-esterifying activity of microsomes from abdominal aortas was approximately the same in all four species.

Cholesterol-esterifying activity of aortas derived from atherosclerosis-resistant pigeons, Show Racer, and atherosclerosis-susceptible pigeons, White Carneau, was examined (Table II). Microsomal cholesterol-esterifying activity was greater in Show Racer females than in Show Racer males. A less convincing but similar sex differential was observed in the White Carneau strain. However, no difences were observed between the two strains.

TABLE I. Cholesterol Esterification by Thoracic and Abdominal Aortic Microsomes Derived from Males of Several Species.[a]

	Incorporation of the palmityl group of palmityl-CoA into cholesteryl esters (pmoles/mg protein)		
	4 min	8 min	16 min
Rat aorta			
Thoracic (T)	34.2 ± 9.2[b]	57.7 ± 15.9	90.8 ± 25.8
Abdominal (A)	10.7 ± 4.0	18.4 ± 6.8	30.7 ± 6.8
T vs A, $P < 0.01$			
Dog aorta			
Thoracic (T)	11.2 ± 1.5	17.6 ± 2.9	21.2 ± 2.9
Abdominal (A)	7.5 ± 1.4	12.8 ± 2.4	24.8 ± 8.6
T vs A, NS			
Rabbit aorta			
Thoracic (T)	10.6 ± 2.0	16.7 ± 2.5	24.2 ± 0.7
Abdominal (A)	7.4 ± 0.5	12.8 ± 1.0	18.5 ± 1.3
T vs A, $P < 0.01$			
Cockerel aorta			
Thoracic (T)	5.6 ± 0.5	9.3 ± 1.7	13.3 ± 2.5
Abdominal (A)	9.3 ± 2.1	15.1 ± 2.2	24.3 ± 4.9
T vs A, $P < 0.01$			

[a] Incubation medium consisted of 0.1 *M* phosphate buffer (pH 7.4) containing 1% albumin, 0.4 μCi[1-^{14}C]palmityl-CoA (sp act, 58 μCi/μmole) and 0.3–0.5 mg microsome. Reaction mixture was shaken at 37° under air. Significance of the difference between the values obtained with thoracic and abdominal aortas was assessed by the analysis of variance.

[b] Mean ± standard deviation of three experiments.

TABLE II. Cholesterol Esterification by Aortic Microsomes from White Carneau and Show Racer Pigeons.[a]

	Incorporation of the palmityl group of palmityl-CoA into cholesteryl esters (pmoles/mg protein)		
	4 min	8 min	16 min
Show Racer			
Male (M)	10.1 ± 0.9[b]	18.7 ± 2.4	25.4 ± 2.4
Female (F)	20.1 ± 8.2	31.9 ± 13.5	43.8 ± 19.8
M vs F, $P < 0.05$			
White Carneau			
Male (M)	11.7 ± 3.8	20.0 ± 5.0	28.2 ± 8.2
Female (F)	15.0 ± 4.4	25.5 ± 5.6	35.0 ± 9.1
M vs F, $P < 0.10$			

Show Racer male vs White Carneau male, NS
Show Racer female vs White Carneau female, NS

[a] Conditions of incubation were the same as described in Table I. Tests of significance were performed using the analysis of variance.

[b] Mean ± standard deviation of three experiments.

Discussion. The dominant cholesterol-esterifying activity in the aortas of rabbits and pigeons is due to acyl-CoA:cholesterol acyltransferase (2, 5). Assuming that the same mechanism is responsible for cholesterol esterification in rat, dog, and chicken aortas, we assayed cholesterol-esterifying activity in all these species using palmityl-CoA as a substrate.

Species vary in their susceptibility to atherosclerosis. For example, the rat and dog are resistant, while the rabbit and chicken are susceptible to atherosclerosis (14). Anatomical localization of atherosclerotic lesions varies; they are more prevalent in the abdominal portions of the aorta in the dog (15) and the rat (16), whereas in the rabbit (17), lesions are found mainly in the thoracic portion of the aorta and in the chicken the lesions are found in both the thoracic and abdominal portions of the aorta with cholesterol-feeding (18). If the magnitude of the cholesterol-esterifying activity of aortas bears a relationship to susceptibility, we would expect that the relative order of cholesterol-esterifying activity would follow the relative order of susceptibility. The results of these experiments did not support this relationship. In fact, in the rat, probably the most resistant of these five species to induction of atherosclerosis, the microsomal cholesterol-esterifying activity was by far the greatest of the animals studied.

We also examined the cholesterol-esterifying activity in aortas of two strains of pigeons, one of which (White Carneau) develops atherosclerosis spontaneously (19). This seems to be a more critical test of the hypothesis; if the peculiar characteristic of the White Carneau is due to a single-gene defect, pathways other than the affected one ought to function similarly in both strains. We found that the cholesterol-esterifying activity was the same in both the White Carneau and the Show Racer. Thus, as with other species studied, the cholesterol-esterifying activity in the aortas of the pigeons could not be related to the susceptibility of these pigeons to atherosclerosis. However, a sex difference in cholesterol-esterifying activity was observed. This unexpected finding does not shed light on mechanisms of susceptibility, since in the pigeon, the two sexes are equally susceptible (19).

The results thus indicate that the cholesterol-esterifying activity of normal aortic tissue does not reflect the predisposition of the tissue to develop atherosclerosis. It is, of course, conceivable that there are differences in inducibility of the microsomal enzyme by cholesterol feeding, but we do not perceive a satisfactory way of testing this possibility.

The most striking finding in these experiments was the very high microsomal cholesterol-esterifying activity in arterial tissue of the rat, which is probably the most atherosclerosis-resistant of the several species involved in this study. This observation demands consideration of an alternative hypothesis: that cholesterol esterification is a protective phenomenon (*i.e.*, that free cholesterol is atherogenic and that its esterification is an adaptive change). We can pose but not further support such a hypothesis. It would be more attractive if all our observations showed an inverse relationship between susceptibility and cholesterol-esterifying activity, but this was not the case.

Summary. A study was undertaken to relate the magnitude of the cholesterol-esterifying activity in aortic microsomes to the susceptibility of species to atherosclerosis and to the propensity of the thoracic and abdominal segments within a species to develop atherosclerosis. The cholesterol-esterifying activity was the greatest in the rat, probably the most resistant to atherosclerosis, and the least in the cockerel, one of the species more susceptible to atherosclerosis. The cholesterol-esterifying activity in the aorta was the same for the dog (atherosclerosis resistant) and the rabbit (atherosclerosis susceptible). Relative cholesterol-esterifying activities of thoracic and abdominal portions of the aorta within a species also did not coincide with the relative susceptibility of these anatomical sites to atherosclerosis. The cholesterol-esterifying activity was examined in the aortas of two genetic strains of pigeons which vary in their susceptibility to atherosclerosis; the enzyme activity in the aorta of the White Carneau (atherosclerosis resistant) was the same as that of the Show Racer (atherosclerosis susceptible). Thus, cholesterol-esterifying activity does not consistently reflect the predisposition of the tissue to develop atherosclerosis.

Pigeon microsomes displayed an unexpected difference between the sexes. In the Show Racer, cholesterol-esterifying activity was significantly greater in the female aortic microsomes than in the male. A similar but smaller trend was apparent in the White Carneau.

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