

Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



A Biochemical Study of Spontaneous Atherosclerosis in Pigeons

HUGH B. LOFLAND, JR. and THOMAS B. CLARKSON

Circ. Res. 1959;7:234-237

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

Copyright © 1959 American Heart Association. All rights reserved. Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circres.ahajournals.org>

Subscriptions: Information about subscribing to Circulation Research is online at <http://circres.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail: journalpermissions@lww.com

Reprints: Information about reprints can be found online at <http://www.lww.com/reprints>

A Biochemical Study of Spontaneous Atherosclerosis in Pigeons

By HUGH B. LOFLAND, JR., PH.D., AND THOMAS B. CLARKSON, D.V.M.

Two breeds of pigeons (White Carneau and Silver King) have been found to have a high incidence of spontaneous atheromatous lesions in the aorta, while two breeds of homing pigeons (Racing Homers and Show Racers) seemed to be resistant to atherosclerosis. The weight of the aorta, and its cholesterol content, paralleled the severity of the disease. The levels of serum cholesterol and phospholipids, and the cholesterol: phospholipid ratio, however, appear to be unrelated to the incidence and severity of atherosclerosis in pigeons. The differences observed among the four breeds of birds showed little correlation with age, sex, diet, or physical activity.

IN A RECENT study made at this institution, it has been demonstrated that certain breeds of pigeons show spontaneous atherosclerotic lesions of the aorta which are strikingly similar to those found in man. Plaques were found most commonly at the distal end of the thoracic aorta, although lesions were encountered along the entire thoracic aorta and occasionally extended into the pectoral arteries. The lesions occur as moderately yellow elevated plaques, from 1 to 10 mm. in diameter, and from 0.5 to 3.0 mm. in thickness. Ulceration and hemorrhage into the plaques were not uncommon. Histochemically, they appeared rich in intracellular and extracellular sudanophilic material, with large deposits of cholesterol and calcium salts in the intima. At least some degree of atherosclerosis occurred in all birds of two of the breeds we have examined, while two other breeds appeared to be relatively free of the disease.*

Spontaneous arteriosclerosis has been said to occur in the dog,¹ cat,² chicken,³ rat,⁴ and rabbit.⁵ However, no laboratory animal, except perhaps the baboon,⁶ exhibits spontaneous atherosclerotic lesions similar to the human lesions. In view of the observations noted above, we thought that the pigeon might be a

convenient and readily available animal for research on this disease. The serum and aorta of birds from atherosclerosis-susceptible and nonsusceptible breeds, were analyzed for certain lipids which have been reported to be related to the atherosclerotic process. In addition, an attempt has been made to estimate the extent or severity of the condition, and to correlate these findings with serum lipid levels.

METHODS

Four breeds of pigeons were used. Racing Homers (RH) were purchased from a nearby racing loft. Show Racers (SR), Silver King (SK), and White Carneau (WC) pigeons were obtained from the Palmetto Pigeon Plant, Sumter, South Carolina. Their dietary background is described below.

A 10 ml. sample of blood was drawn from the alar vein without anesthesia, and the bird was killed by the intravenous injection of pentobarbital sodium. The entire thoracic aorta was removed, cleaned of adhering tissue, and opened longitudinally. The area of the rectangular section of tissue was calculated from its length and width. The length and width of the plaques, which were clearly visible without magnification, were also measured, and their total area was calculated by assuming a rectangular shape for each plaque. The percentage of that area of the segment of aorta occupied by plaques was indicated as "atherosclerotic index." While such type of measurement does not take into account the thickness of the plaques, which occasionally almost occluded the lumen of the vessel, it gives at least a rough approximation of the degree to which the aorta is involved with atherosclerotic lesions.

*A complete description of these findings is being prepared for publication by Clarkson, T. B., Prichard, R. W., Netsky, M. G., and Lofland, H. B.

From the Department of Biochemistry, and The Vivarium, The Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N.C.

Supported by grants from the United Medical Research Fund of North Carolina, the North Carolina Heart Association, and the U.S. Atomic Energy Commission (Contract AT-(40-1)-1638).

Received for publication October 27, 1953.

TABLE 1.—*Atherosclerotic Index, and Lipids of the Aorta and Serum in Pigeons of Various Breeds**

Breed†	Sex and no. of birds	Atherosclerotic index‡	Aorta		Blood serum					
			Wt.§ (mg.)	Cholesterol (mg./Gm.)	Total cholesterol (mg. %)	Free cholesterol (mg. %)	Ester cholesterol (mg. %)	Phospholipids (mg. %)	Ratio, cholesterol: phospholipids	
Racing Homers (4.4±0.5)	M	10	0.48	41.3	3.69	361.1	130.6	240.6	718.7	0.48
			±0.27	±1.9	±0.19	±25.8	± 7.3	±26.5	±22.0	
	F	3	0	36.7	4.13	302.9	121.3	181.3	679.3	0.44
				±2.7	±0.06	±36.9	±12.2	±26.3	±60.8	
Show Racers (6.8±0.3)	M	7	1.47	43.5	5.61	363.9	118.7	245.1	573.4	0.63
			±0.56	±1.3	±0.84	±39.5	±11.5	±29.0	±51.7	
	F	7	1.24	41.0	5.29	317.5	111.7	205.9	537.7	0.59
			±1.05	±2.1	±0.57	±10.9	± 9.6	± 4.3	±40.1	
Silver King (7.4±0.3)	M	12	11.98	67.8	14.47	327.9	101.7	226.4	506.3	0.64
			±1.86	±3.4	±2.21	±16.2	± 4.4	±12.5	±36.0	
	F	12	11.45	64.1	8.28	430.0	176.4	253.6	913.5	0.47
			±1.35	±2.5	±0.94	±18.8	±13.8	±14.7	±107.9	
White Carneau (7.8±0.1)	M	12	9.30	68.0	12.92	372.1	111.7	260.4	523.8	0.70
			±1.84	±2.8	±2.94	±16.0	± 4.9	±16.7	±21.1	
	F	12	13.23	64.3	10.97	383.2	149.8	242.5	721.2	0.54
			±1.55	±2.2	±1.22	±18.8	±10.3	±15.5	±69.4	

*Average values, followed by the standard errors of the means.

†Figures in parentheses are average age in years.

‡Per cent of surface area of aorta occupied by plaques.

§Wet weight.

After these measurements were made the aortas were rapidly weighed and immediately minced under alcohol. Lipids were extracted with hot alcohol-ether and purified with chloroform.⁷ Aorta cholesterol was determined on the chloroform extract by a modification of the procedure described by Abell, Levy, Brodie, and Kendall.⁸

Blood lipid determinations were carried out on aliquots of an alcohol-ether extract of serum, prepared by the method of Boyd.⁹ Total serum cholesterol was determined according to Abell et al., and free cholesterol was determined after precipitation as the digitonide. Values for ester cholesterol were obtained by difference. Lipid phosphorus was determined, after incineration, by the method of Fiske and SubbaRow,¹⁰ and the values for serum phospholipids (lipid phosphorus × 25) were calculated.

RESULTS

From the values for the "atherosclerotic index" given in table 1, it is apparent that in the SK and WC pigeons there was extensive plaque formation, 9 to 13 per cent of the aorta being occupied by plaques. It is of interest that all of the birds belonging to these breeds had some plaques in the aorta. On the other hand, both breeds of homing pigeons (RH and

SR) appear to be resistant to the disease. The much lower incidence and severity of atherosclerotic lesions in these breeds is paralleled by the much lower weight and cholesterol content of the aortas. While the SR pigeons had a significantly higher ($p < .01$) aorta cholesterol than RH birds, neither breed approached the values obtained for SK and WC pigeons. The fact that the RH pigeons were considerably younger than the SR birds could perhaps explain the difference between these two groups. The Racing Homers were included in this study in order to obtain information about birds that were genetically related to the Show Racers, but that had been exercised more actively. These birds were obtained from a racing loft, were raised on a commercial pigeon ration, and ranged considerably in age. Accordingly, we realize that the values obtained for this group are not directly comparable with those of the other three groups, and the data are to be regarded as merely suggestive.

There was no apparent correlation between the degrees of atherosclerotic damage and

serum lipid levels. The average values for total, free, and ester cholesterol were practically identical in the four breeds of birds. Likewise, significant differences were not observed in the levels of serum phospholipid and in the cholesterol:phospholipid ratios.* It would appear that in pigeons, serum cholesterol level is not a reliable index of the extent of aortic atherosclerosis, just as has been noted in baboons by Gillman,⁸ and in human beings, for example, by Landé and Sperry,¹¹ and by Faber.¹²

DISCUSSION

During recent years, much attention has been focussed on the possible effects of various factors (age, sex, diet, physical activity, etc.) on atherogenesis. It is of interest to examine the disease in pigeons as it relates to each of these factors.

Age. Among the RH pigeons, lesions were found only in one three-year-old and one five-year-old bird. The pigeons of this group ranged in age from one to eight years, with an average age considerably lower than that of the other three groups. However, the SR birds, which showed a low susceptibility to atherosclerosis, were of approximately the same age as the SK and WC, which were highly susceptible.

Accordingly, age alone is not responsible for the differences in the incidence of the disease in these three breeds of pigeons. Levi¹³ states that it is not uncommon for pigeons to live from 15 to 20 years, and the 6 to 7 year old birds are often actively reproducing. It seems likely that the SR, SK, and WC birds in this series should be considered "middle-aged."

Diet. From the time of leaving the nest, the SR, SK, and WC pigeons were housed in wire cages at the Palmetto Pigeon Plant, and were fed a ration consisting of 40 per cent whole corn, 20 per cent kaffir corn, 20 per cent field peas, 20 per cent wheat, and supple-

mented with a mineral mixture.* In such a diet a deficiency of unsaturated fatty acids, or of other dietary factors, is unlikely. At any rate, since the SR birds consumed the same ration for an equal period of time, observed differences in the susceptibility to the disease could not be ascribed to diet.

Physical Activity. Exercise has been suggested as a factor in the development of human atherosclerosis.¹⁴ For this reason, the group of Racing Homers were included in this study. While the exact amount of exercise given to the RH birds is not known, they were active flying birds obtained from a racing loft. On the other hand, pigeons of another breed, SR, were decidedly more sedentary, having been raised in commercial squab production cages, and were never flown outside the pens. In both breeds of homing pigeons the levels of serum cholesterol were almost identical, while the incidence of atherosclerosis was very low compared to the other two breeds, SK and WC, which were maintained under the same conditions as the SR birds.

Sex. A striking sex difference in the incidence of atherosclerosis has been reported in human beings,¹⁵ although the extent of this difference varies with race and perhaps with occupational habits. From table 1 it appears that the values for the "atherosclerotic index" and the aorta cholesterol content in the two sexes are not significantly different. While sex differences can be seen in the serum lipid values of some of the breeds of pigeons, there seems to be no consistent pattern for such differences. Furthermore, the same conclusions are reached when the averages for each group are calculated with as without regard to sex.

It appears that none of the factors discussed above, which have been claimed to be related to atherosclerosis in human beings, explains sufficiently the differences observed

*Only when the average phospholipid levels are calculated for the entire RH and SR groups without regard to sex do significant differences appear between these two groups.

*Composed of 45 per cent medium-sized crushed oyster shell, 40 per cent limestone grit, 5 per cent ground bone, 5 per cent ground limestone, 4 per cent salt, 1 per cent Venetian red, and prepared and fed at the Palmetto Pigeon Plant, Sumter, S.C. Such mixtures are usually termed "health grit" by pigeon growers.

among the four breeds of pigeons studied. It might be tentatively suggested that atherogenesis in pigeons results from an alteration of some metabolic factor which is genetically controlled.

SUMMARY

Four breeds of pigeons were examined for atheromatous lesions in the aorta, and analyses for serum and aorta lipids were made. White Carneau and Silver King pigeons were found to have a high incidence, while two breeds of homing pigeons (Racing Homers and Show Racers) appear to be resistant to atherosclerosis. The incidence and severity of the disease seemed to be unrelated to the levels of serum total, free, and ester cholesterol, as well as to the values for total phospholipids and for the cholesterol:phospholipid ratio in the serum. On the other hand, the weight of the aorta and its cholesterol content parallel the severity of the disease. There was no indication that the differences observed might be due to differences in age, sex, diet, or physical activity.

ACKNOWLEDGMENT

The authors are indebted to Mr. Wendell Levi, of the Palmetto Pigeon Plant, for his interest and help in selecting the pigeons used in this study, and to Mr. William Spencer and Miss Katherine King for valuable technical assistance.

SUMMARIO IN INTERLINGUA

Quatro racias de columba esseva examine con respecto al presentia de lesiones atheromatose in le aorta. Analyses pro lipidis in le sero e le aorta esseva executate. Esseva trovate que le racias White Carneau e Silver King ha un alte incidentia de atherosclerosis, durante que le messageros Racing Homers e Show Racers pare esser resistent a ille condition. Le incidentia e le grado de severitate del morbo esseva apparentemente sin relation con le nivellos seral de cholesterol total, libere, e esterice e etiam con le valores seral de phospholipidos total e del proportion de cholesterol a phospholipido. Del altere latere, le peso del aorta e le contento de cholesterol in le aorta es parallel al severitate del morbo.

Esseva notate nulle ration pro supponer que le differentias observate es possibilmente relationate a differentias de etate, sexo, dieta, o activitate physic.

REFERENCES

1. LINDSAY, S., CHAIKOFF, I. L., AND GILMORE, J. L.: Arteriosclerosis in the dog: Spontaneous lesions in the aorta and the coronary arteries. *Arch. Path.* **53**: 231, 1952.
2. —, AND —: Arteriosclerosis in the cat: Naturally occurring lesions in the aorta and the coronary arteries. *Arch. Path.* **60**: 29, 1955.
3. DAUBER, D. V.: Spontaneous arteriosclerosis in chickens. *Arch. Path.* **38**: 46, 1944.
4. HUMPHRIES, E. M.: The occurrence of atheromatous lesions in the coronary arteries of rats. *Quart. J. Exper. Physiol.* **42**: 96, 1957.
5. BRAGDON, J. H.: Spontaneous atherosclerosis in the rabbit. *Circulation* **5**: 641, 1952.
6. GILLMAN, J., AND GILBERT, C.: Atherosclerosis in the baboon (*Papio ursinus*): Its pathogenesis and etiology. *Exper. Med. & Surg.* **15**: 181, 1957.
7. ARTOM, C.: Phospholipid metabolism in denervated muscle. *J. Biol. Chem.* **139**: 953, 1941.
8. ABELL, L. L., LEVY, B. B., BRODIE, B. B., AND KENDALL, F. E.: A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195**: 357, 1952.
9. BOYD, E. M.: The extraction of blood lipids. *J. Biol. Chem.* **144**: 223, 1936.
10. FISKE, C. H., AND SUBBAROW, Y.: The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375, 1925.
11. LANDÉ, K. D., AND SPERRY, W. M.: Human atherosclerosis in relation to the cholesterol content of the blood serum. *Arch. Path.* **22**: 301, 1936.
12. FABER, M.: The cholesterol content of the human aorta in relation to the serum cholesterol content. *Acta med. scandinav.* **125**: 418, 1946.
13. LEVI, W. M.: *The Pigeon*. Sumter, S.C., Levi Publishing, 1957, p. 272.
14. MORRIS, J. N., HEADY, J. A., RAFFLE, R. A. B., ROBERT, C. G., AND PARKS, J. W.: Coronary heart disease and physical activity of work. *Lancet* **2**: 1053, 1111, 1953.
15. FRIEDMAN, M., AND ROSENMAN, R. H.: Comparison of fat intake of American men and women: Possible relationship to incidence of clinical coronary artery disease. *Circulation* **16**: 339, 1957.