

Naturally Occurring Atherosclerosis in Turkeys* (33626)

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The purpose of this paper is to report on a comparison of the extent of coronary artery and aortic atherosclerosis, blood pressures, and serum cholesterol levels in Broad Breasted Bronze and Beltsville White breeds of turkeys from 1 day through 24 weeks of age.

Aortic rupture in commercial flocks of Broad Breasted Bronze Turkeys was first reported in 1952 (1). In 1955, aortic atherosclerosis was described in turkeys dying from aortic rupture as well as in those in apparent good health (2). Coronary atherosclerosis was reported in turkeys in 1965 (3). It has been suggested that hypertension might be responsible for aortic rupture in turkeys (4). Serum cholesterol levels reportedly increase with age in turkeys (5).

Materials and Methods. All turkeys used in this study were hatched in January, 1963 at the J. E. Davis Turkey Farm, Dazell, South Carolina. They were maintained by the Davis Turkey Farm until acquired for autopsy. The birds were fed a commercial turkey ration consisting of 28% protein from 0-8 weeks, 20% from 8-16 weeks, and 16% from 16-24 weeks. Only males were used and each group consisted of 15 birds except at week 24 when 5 birds were studied. The ages of the groups studied were: 1 day and 2, 4, 6, 8, 10, 12, 16, and 24 weeks.

The birds were restrained on their backs for blood pressure determinations. A local anesthetic was infiltrated into the ventral aspect of the neck near the ramus of the mandible, and a hood was placed over the bird's head. With gentle handling, the bird remained quiet during surgical exposure of the carotid artery. The artery was cannulated

using an 18-gauge needle with polyethylene tubing containing heparinized saline attached to a Statham transducer (Stratham Hato Rey, Puerto Rico, 10 V 0-75 cm Hg, model p23Gc) which was connected to a Sanborn Polyviso recorder (Model 67-1200, Sanborn Company, Cambridge, Massachusetts) for blood pressure determinations. After blood pressure determinations, the blood was allowed to flow freely through the polyethylene tubing to clear the heparinized saline prior to taking the blood sample for serum cholesterol determination. Serum cholesterol levels were determined using an automated method (6).

After determination of blood pressure and blood collection, the birds were killed with an euthanizing dose of pentobarbital sodium. The body weight was recorded and the heart and aorta were removed. Heart weights were recorded and the aorta was opened longitudinally and an atherosclerosis index (A. I.) determined (percentage of the intimal surface with grossly visible plaque). The opened aortas were then stained with Sudan IV using the method of Holman *et al.* (7) and a second A. I. calculated (percentage of the intimal surface staining with Sudan IV). Body and heart weights were recorded as normative data for future reference.

The extent of coronary artery atherosclerosis was determined using the method of Clarkson *et al.* (8) Blocks were taken from the thoracic and abdominal aorta for frozen section and paraffin sectioning. Frozen sections were stained with Sudan IV hematoxylin, alcian blue, and aldehyde fuchsin. Paraffin sections were stained with hematoxylin and eosin.

The gross and microscopic characteristics of aortic and coronary atherosclerosis of Broad Breasted Bronze (B.B.B.) turkeys have been described previously (3). The gross and microscopic arterial lesions of the

* This study was supported by a grant from the National Institutes of Health (HEO8996).

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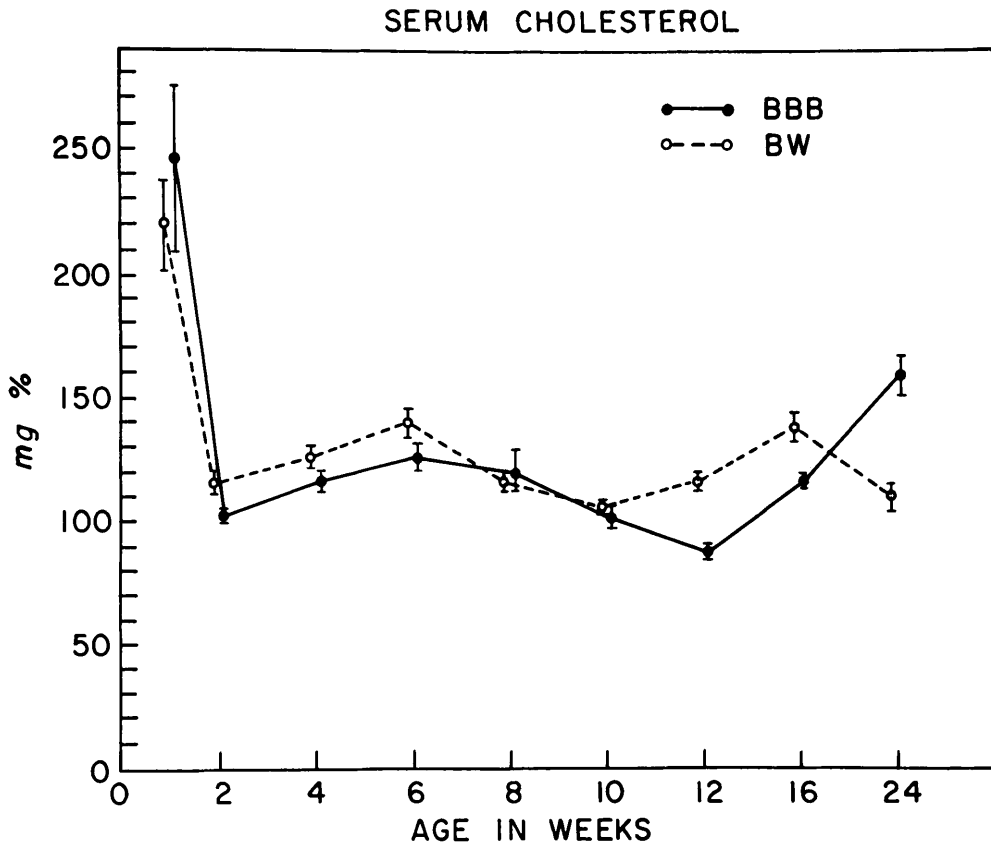


FIG. 1. Serum cholesterol values for Broad Breasted Bronze (BBB) and Beltsville White (BW) turkeys from 1 day to 24 weeks of age.

Beltsville White (B. W.) turkeys used in this study resembled those of B.B.B. turkeys.

Results. Neither breed had gross aortic lesions before 6 weeks of age; however, after Sudan IV staining sudanophilic areas could be seen at the coeliac branch. All birds 6 weeks and older had gross aortic intimal sudanophilia. By 6 weeks of age some birds of both breeds had small plaques visible without stain.

The B.B.B. turkeys had significantly more aortic intimal sudanophilia (A. I. = 28%) at 16 weeks of age than did the B. W. turkeys (A. I. = 13%). The significance of the difference in these means was determined using Student's *t* test and the probability was less than 0.002. The same was true in the 24-week-old birds; A. I. for B.B.B = 26% and B. W. = 8%, $p < .001$.

The earliest microscopic changes seen were

thickening of the intima and disruption of the internal elastic lamina. At 16 weeks of age stainable lipid became a significant part of the lesion. Most sections were positive for alician blue, presumably indicating increased amounts of acid mucopolysaccharide. Densely basophilic material was seen in the hematoxylin-eosin-stained sections of some of the plaques and was presumed to be calcium salts. Cholesterol crystal clefts, hemorrhage, vascularization, and ulceration were not seen in any of the plaques.

Coronary artery atherosclerosis has been reported in B.B.B turkeys (3). In this study it was seen in a few of the intramyocardial coronary arteries as early as 10 weeks among some of the B.B.B. turkeys. Of all the B.B.B turkeys only 3 had coronary atherosclerosis and this involved only a very few of the small intramyocardial arteries. There was

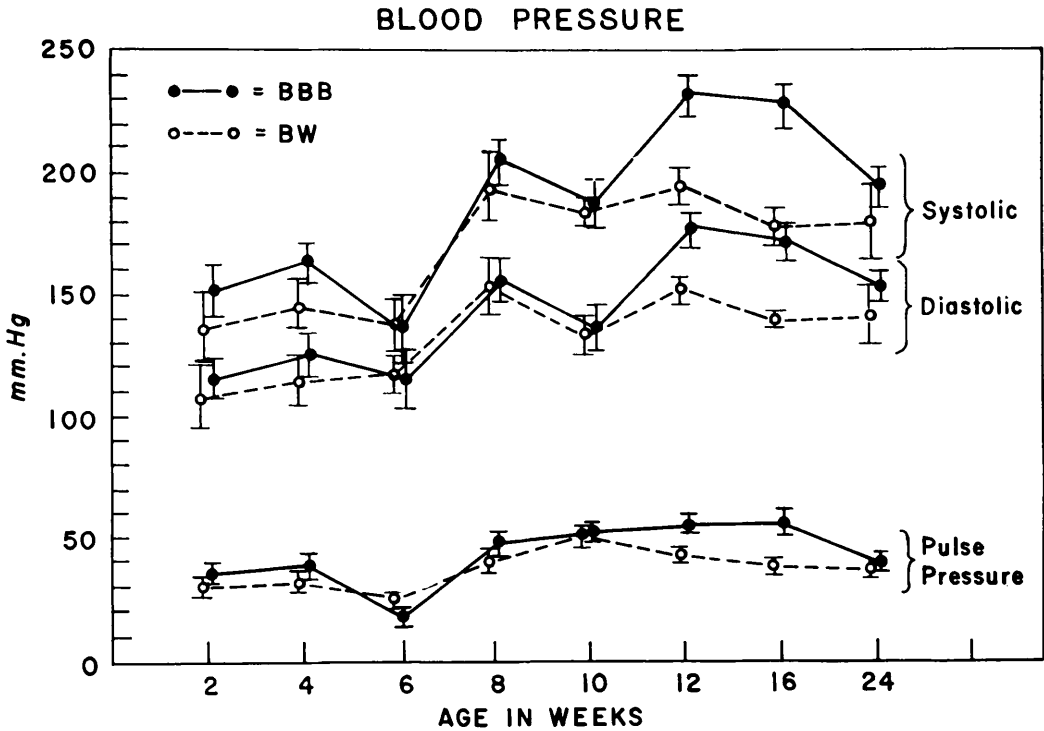


FIG. 2. Blood pressure values for Broad Breasted Bronze (BBB) and Beltsville White (BW) turkeys from 2 to 24 weeks of age.

medial fat accumulation in 2 of the birds with coronary atherosclerosis.

Serum cholesterol levels of both breeds were significantly higher in the day-old birds than any other age group ($p = < .001$). From 2 weeks of age through 16 weeks of age the serum cholesterol values of the two breeds followed the same trend. However, between the 16- and 24-week-old groups the cholesterol values of the B.B.B. increased while those of the B.W. decreased.

The cholesterol values for both breeds and the age groups studied are shown in Fig 1.

The earliest blood pressure measurements were made on 2-week-old birds. Among B.B.B. turkeys both systolic and diastolic blood pressures were significantly higher at 24 weeks than at 2 weeks of age ($p = < .01$). The blood pressures of both breeds at the different ages are shown graphically in Fig. 2.

Heart rates decreased significantly among birds of both breeds between 2 and 24 weeks of age ($p = < .01$). Heart rates are shown

graphically in Fig. 3. Table I details the heart rates and blood pressures of both breeds of turkeys at each age studied.

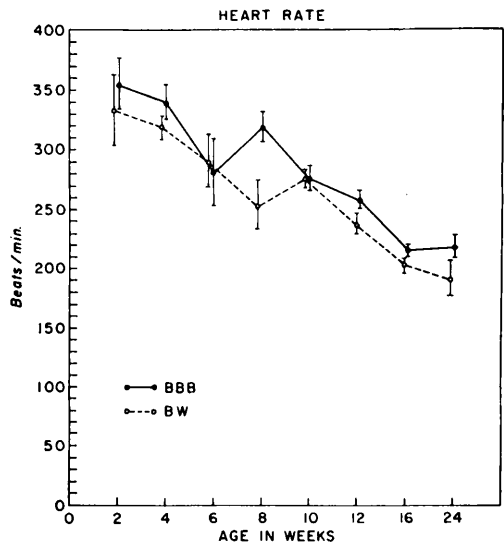


FIG. 3. Heart rate values for Broad Breasted Bronze (BBB) and Beltsville White (BW) turkeys from 2 to 24 weeks of age.

Table II shows heart and body weights for both breeds at each age studied. The increase in body weight between 1 day to 24 weeks of age in turkeys is striking.

Discussion. The study reported here was designed to study blood pressure, serum cholesterol levels, and coronary and aortic atherosclerosis in two breeds of turkeys. Breed differences have been reported in susceptibility to atherosclerosis between the White Carneau and Show Racer pigeons (9), a finding which has served as a useful tool in the study of atherosclerosis.

Breed differences were noted between the B.B.B. and the B.W. turkeys in the extent of aortic atherosclerosis with the B.B.B. turkeys having significantly more involvement. The number of birds with coronary lesions was so small that no breed difference could be detected.

The systolic blood pressure of birds in the study was considerably lower than reported previously for the B.B.B. turkey, the order of magnitude being about 100 mm. Hg. (10). No report in the literature could be found on the blood pressure of the B.W. turkey. It has been reported that the blood pressure of turkeys is lower in the summer months (10); however, all blood pressure measurements in this experiment were done during the winter and early spring.

The serum cholesterol levels reported here are much lower than those previously reported, which were: 148 mg/100 ml at 8 weeks, 180 mg/100 ml at 12 weeks, and 249 mg/100 ml at 16 and 24 weeks of age (5). The highest levels in this study were 250 mg/100 ml at day 1, with a significant fall by 2 weeks of age to about 100 mg/100 ml and never exceeding 160 mg/100 ml in the B.B.B. male turkeys in any of the other age groups examined.

Although the atherosclerotic lesions were small and uncomplicated it should be emphasized that the oldest birds studied were 24 weeks of age. Turkeys live to be considerably older than this and the progression of the disease past 24 weeks is not known. Turkeys grown for commercial purposes are not generally kept beyond 1 year of age. This study was designed to study older groups of

TABLE I. Heart and Blood Pressure.

Age (weeks)	Heart rate ^a		Systolic pressure ^b		Diastolic pressure ^b		Pulse pressure ^b	
	B.B.B.	B.W.	B.B.B.	B.W.	B.B.B.	B.W.	B.B.B.	B.W.
	2	357 ± 22.02	333.3 ± 31.94	153 ± 10.76	138 ± 14.62	117 ± 8.58	108 ± 12.24	36 ± 2.85
4	340 ± 15.78	319 ± 9.99	165 ± 7.51	146 ± 10.62	126 ± 9.91	115 ± 9.44	39 ± 4.44	30 ± 3.37
6	283 ± 27.37	290 ± 22.33	137 ± 14.81	139 ± 11.45	117 ± 12.28	118 ± 10.34	20 ± 2.67	24 ± 2.18
8	320 ± 12.34	252 ± 19.10	206 ± 10.97	194 ± 14.51	157 ± 8.68	155 ± 11.76	49 ± 4.65	39 ± 3.57
10	277 ± 11.83	279 ± 8.6	190 ± 10.1	185 ± 6.95	137 ± 9.98	135 ± 8.52	53 ± 3.63	50 ± 3.6
12	259 ± 8.16	236 ± 7.61	233 ± 8.67	196 ± 6.62	177 ± 6.04	154 ± 5.07	55 ± 4.13	42 ± 2.76
16	216 ± 4.86	203 ± 6.7	228 ± 9.17	179 ± 5.34	172 ± 5.29	140 ± 3.88	57 ± 5.09	38 ± 1.65
24	219 ± 9.65	189 ± 15.72	194 ± 8.48	180 ± 15.42	154 ± 6.51	143 ± 12.44	41 ± 4.21	38 ± 3.57

^a Expressed as beats per minute followed by the standard deviation.

^b Expressed as mm Hg followed by the standard deviation.

TABLE II. Heart and Body Weights.*

Age	Heart weight (g)		Body weight (g)	
	B.B.B.	B.W.	B.B.B.	B.W.
1 day	.58 ± .016	.52 ± .013	78.6 ± 2.27	73.64 ± 1.92
2 weeks	2.00 ± .057	2.13 ± .086	293.7 ± 8.53	273.80 ± 7.13
4 weeks	4.85 ± .133	4.82 ± .204	767.0 ± 21.86	851.9 ± 22.68
6 weeks	8.51 ± .270	7.61 ± .232	1620.9 ± 28.51	1439.0 ± 47.00
8 weeks	12.80 ± .52	11.00 ± .29	2192.7 ± 47.47	2039.3 ± 30.46
10 weeks	17.30 ± .689	16.60 ± .62	3417.1 ± 88.19	3264.0 ± 65.74
12 weeks	21.29 ± .73	19.68 ± .52	4660.0 ± 95.5	4140.0 ± 84.96
16 weeks	33.07 ± 1.25	24.95 ± .53	6831.3 ± 82.47	5793.3 ± 114.00
24 weeks	27.40 ± 4.55	23.09 ± 2.84	6720.0 ± 839.65	5726.7 ± 615.35

* Expressed as means for the group followed by the standard deviation.

birds; however, the B.W. birds were marketed at 25 weeks of age because of a disease outbreak, thus making further breed comparison impossible.

In comparison with squirrel monkeys and White Carneau pigeons in which atherosclerosis occurs naturally, turkeys appear to be of lesser value as animal models for the study of atherosclerosis. Aggravation of coronary and aortic atherosclerosis is readily accomplished by the addition of 0.5% cholesterol to the diet in White Carneau pigeons and squirrel monkeys (*Saimiri sciureus*) (11, 12). Gross intimal sudanophilia and raised plaques occur at an earlier age in turkeys than in either the pigeon or squirrel monkey. This might prove useful in testing drugs which are thought to be of value in preventing or regressing the atherosclerotic process.

Summary. Coronary artery and aortic atherosclerosis was studied in the Broad Breasted Bronze and Beltsville White breeds of turkeys from 1 day of age through 24 weeks of age. Serum cholesterol levels and blood pressures were reported. The usefulness of turkeys as an animal model for the study of atherosclerosis was compared to the White Carneau pigeon and squirrel monkey (*Saimiri sciureus*).

and Jerstad, A. C., Proc. 89th Ann. Meeting Am. Assoc., Atlantic City, p. 280 (1952).

2. Carnaghan, R. B. A., Vet Record 67, 568 (1955).

3. Roberts, J. C., Jr. and Straus, R., in "Comparative Atherosclerosis, The Morphology of Spontaneous and Induced Atherosclerotic Lesions in Animals and Its Relation to Human Disease," p. 59. Harper (Hoerber), New York (1965).

4. Weiss, H. S. and Sheahan, M., Am. J. Veterinary Res. 19, 209 (1958).

5. Ringer, R. K., Conf. on Use of Reserpine in Poultry Production, 2nd Ciba Pharmaceutical Products, Inc., (1960).

6. Methodology N-24, Technicon Instrument Company, Chauncey, New York.

7. Holman, R. L., McGill, H. C., Jr., Strong, J. P., and Geer, J. C., Lab. Invest. 7, 1:42 (1958).

8. Clarkson, T. B., Prichard, R. W., and Lofland, H. B., Circulation Res. 11, 400 (1962).

9. Clarkson, T. B. Prichard, R. W., Netsky, M. G., and Lofland, H. B., Arch. Pathol. 68, 143 (1959).

10. Ringer, R. K., Conf. on Use of the Tranquilizing Anti-hypertensive Agent. Serpasil in Animals and Poultry Production, 1st Ciba Pharmaceutical Products, Inc. (1959).

11. Clarkson, T. B. and Lofland, H. B., Circulation Res. 9, 106 (1961).

12. Middleton, C. C., Clarkson, T. B., Lofland, H. B., and Prichard, R. W., Arch. Pathol. 83, 145 (1967).

1. Durrell, W. B., Pomeroy, B. S., Carr, W. W.,

Received Oct. 16, 1968. P.S.E.B.M., 1969, Vol. 130.