WE HAVE previously reported that Silver King (SK), Autosexing King (AK), and White Carneau (WC) pigeons of the Palmetto Strain* spontaneously develop atherosclerosis on grain diets, while Racing Homers (RH) and Show Racers (SR) are resistant to the disease. Biochemical studies showed a lack of correlation between "atherosclerotic index" and the levels of serum lipids, and no differences in the levels of serum lipids could be demonstrated between the susceptible and resistant breeds.

This report deals with a comparison of the effects of feeding cholesterol-fat diets to genetically resistant pigeons (Racing Homers) and to pigeons susceptible to spontaneous atherosclerosis (White Carneau). Evidence is presented to indicate that the pigeon's susceptibility to spontaneous atherosclerosis is indicative of its susceptibility to cholesterol-fat-induced atherosclerosis, and that morphologically different lesions are produced in these two breeds.

Methods

All pigeons used in these experiments were obtained from the Palmetto Pigeon Plant, Sumter, South Carolina, where the different breeds are maintained under identical conditions of housing and diet. White Carneau pigeons were chosen as the atherosclerosis-susceptible breed, Racing Homers as the resistant breed, and Autosexing Kings as a breed thought to be genetically intermediate. The family histories of these breeds of pigeons are pertinent to this study. The entire White Carneau population at the Palmetto Pigeon Plant was derived from four pair obtained in 1916. These birds were maintained as a closed colony until 1954 when some outerrosses were made to White Kings. The Autosexing Kings were produced at the Palmetto Pigeon Plant by repeated back-crossings, using Silver Kings (an atherosclerosis-susceptible breed) and Blue Homers as the basic breeds. Racing Homers were developed about 150 years ago from several breeds of pigeons not closely related to the other breeds mentioned.

Six-week-old birds of both sexes were used. These birds were maintained for a period of six to seven months on a diet of Purina Pigeon Pellets which had been coated with crystalline cholesterol dissolved in lard to give a final concentration of 1 per cent of the diet. Control birds of each breed were fed the pigeon pellets alone. At the end of the experimental period, the pigeons were sacrificed, and the serum cholesterol, prevalence of atherosclerosis, and "atherosclerotic index" were determined as previously described. The aortae were then weighed, minced under alcohol, extracted with alcohol and alcohol-ether, and purified with chloroform, as described by Artom. Cholesterol determinations on the chloroform extracts of aortae and directly on the blood serum were performed using the method of Abell et al.

All tissues for histological studies were fixed in 10 per cent neutral formalin. Portions of the aortae of all three breeds and the hearts from the White Carneau and Racing Homer groups were taken for histological study.

Sections of the aortae were made either by the carbowax method of Zunighe et al. or with the freezing microtome. The sections were stained with Oil Red O and counterstained with hematoxylin. In addition, some were stained with aldehyde fuschin for elastic tissue and Mallory for connective tissue.

Three blocks from the left ventricular wall, including the septum, were taken from each heart for study. Each block was then cut in step sections so that five sections were examined from each block, after staining with Oil Red O. The prevalence of coronary atherosclerosis was estimated by determining the per cent of the coronary arteries seen which contained atherosclerotic lesions. The severity of coronary atherosclerosis was

*The Ralston Purina Company, St. Louis, Missouri.
Table 1

Effects of Feeding Cholesterol to Various Breeds of Pigeons

| Breed            | Age when sacrificed (months) | Number of birds | Dietary treatment | Prevalence of lesions in aorta (per cent) | Atherosclerotic index (per cent) | Cholesterol (mg./Gm.) | Serum (mg.%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>White Carneau</td>
<td>7.5</td>
<td>14</td>
<td>Cholesterol-fed</td>
<td>100.0</td>
<td>22.4 ± 6.1</td>
<td>16.6 ± 3.1</td>
<td>1424. ± 327</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>17</td>
<td>Control</td>
<td>17.6</td>
<td>0.2 ± 0.04</td>
<td>5.0 ± 0.2</td>
<td>452. ± 26</td>
</tr>
<tr>
<td>Racing Homers</td>
<td>6</td>
<td>11</td>
<td>Cholesterol-fed</td>
<td>9.1</td>
<td>0.27 ± 0.2</td>
<td>6.1 ± 0.7</td>
<td>1432. ± 274</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>4.3 ± 0.2</td>
<td>442. ± 24</td>
</tr>
<tr>
<td>Autosexing King</td>
<td>6</td>
<td>10</td>
<td>Cholesterol-fed</td>
<td>50.0</td>
<td>4.1 ± 2.2</td>
<td>7.2 ± 1.6</td>
<td>1464. ± 199</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>2.4 ± 0.1</td>
<td>404. ± 40</td>
</tr>
</tbody>
</table>

*Average values, followed by standard errors of the means.
†Cholesterol added to commercial pigeon pellets to a concentration of 1 per cent of the diet.
‡Commercial pigeon pellets.

Table 2

Effect of Cholesterol Feeding on Coronary Atherosclerosis in White Carneau Pigeons

<table>
<thead>
<tr>
<th>Number of birds</th>
<th>Dietary treatment</th>
<th>Sex</th>
<th>Number of coronary arteries seen</th>
<th>Prevalence of lesions† (per cent)</th>
<th>Severity of lesions‡ (per cent)</th>
<th>Aortic index§ (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Cholesterol-fed</td>
<td>Female</td>
<td>504</td>
<td>20.0 (0.42)</td>
<td>17.9 (0.38)</td>
<td>19.6 (7.84)</td>
</tr>
<tr>
<td>5</td>
<td>Cholesterol-fed</td>
<td>Male</td>
<td>320</td>
<td>30.0 (0.07)</td>
<td>29.2 (0.03)</td>
<td>26.9 (8.51)</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>Female</td>
<td>235</td>
<td>1.9 (0.68)</td>
<td>0.08 (0.54)</td>
<td>0.4 (0.6)</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>Male</td>
<td>337</td>
<td>0.3 (0.17)</td>
<td>0.13 (0.06)</td>
<td>0.0 (0.06)</td>
</tr>
</tbody>
</table>

*Average values, with the range of values in parentheses.
†Per cent of the arteries seen which contained atherosclerotic plaques.
‡Average per cent of the lumen involved in those arteries having atherosclerotic plaques.
§Severity of aortic disease.

determined by estimating the per cent of the lumen involved in those coronary arteries having atherosclerotic lesions.

Results and Discussion

The effect of cholesterol-fat diets on aortic atherosclerosis and serum cholesterol is presented in table 1. The levels of serum cholesterol were similar in all three breeds. It can be seen that the atherosclerotic index of the White Carneau was markedly affected by the added cholesterol, while the Racing Homers were only slightly affected. It is of interest that the Autosexing Kings were intermediate in their response to the atherogenic diet, and to recall that they were derived from backcrossings between a susceptible and a resistant strain. Furthermore, these differences in prevalence and severity occurred even though there were no significant differences in the total serum cholesterol at the termination of the experiments. The levels of aorta cholesterol closely paralleled the atherosclerotic index in all cases.

Racing Homers were found to be resistant to cholesterol-induced coronary atherosclerosis. Lesions were found in only one of the eleven birds which had been fed cholesterol. On the other hand, cholesterol feeding markedly accelerated the development of coronary lesions in the White Carneau as shown in table 2. It should be pointed out that spontaneous lesions in the susceptible breed rarely appear before nine months of age.

It is of interest that the prevalence and
severity of coronary atherosclerosis appear to be closely correlated. However, no correlation was seen between the severity of the aortic and coronary disease in the White Carneau pigeon. There was a suggestion of less severe coronary atherosclerosis in females than males, although the number of birds examined was probably too small to allow definite conclusions in this regard. It should also be emphasized that pigeons do not become sexually mature until 5 to 6 months of age, so that there was a relatively short time for estrogen to have influenced the course of the disease.

Histological studies of coronary and aortic lesions revealed morphological differences among the three breeds. Racing Homers and Autosexing Kings developed only "pure atheroma" or "fatty streaks" with no intimal connective tissue proliferation. The lipid appeared to be exclusively extracellular. Figure 1 is typical of the "pure atheromata" seen in the coronary arteries and aortae of the resistant breed. No morphological difference was apparent between the aortic and coronary lesions.

Figure 2 (upper and lower) is typical of the connective tissue-complicated lesions seen in the coronary arteries and aortae of the susceptible breeds, again with no morphological difference between aortic and coronary lesions. All of the lesions studied in the White Carneau were complicated by connective tissue proliferation and appeared to contain intracellular as well as extracellular lipid.
ATHEROSCLEROSIS IN PIGEONS

The differences in the type of lesion seen in pigeons susceptible and resistant to atherosclerosis are noteworthy. McGill and his co-workers recently established that no correlation existed between the prevalence and extent of "pure atheromata" or "fatty streaking" and death due to myocardial infarction in man, whereas a positive correlation existed between the extent of connective tissue proliferation and complicated atherosclerotic lesions and death due to myocardial infarction.

Summary

Cholesterol-fat diets are markedly more atherogenic when fed to a strain of pigeons susceptible to spontaneous atherosclerosis, as compared to a strain resistant to the disease. A breed of pigeons intermediate in susceptibility was found to be intermediate in their response to a high cholesterol-high fat diet. Serum cholesterol levels were similar among the three breeds.

The White Carneau pigeons developed lesions complicated by connective tissue proliferation while the Autosexing Kings and Racing Homers developed merely "pure atheroma" or "fatty streaks."

References
Effects of Cholesterol-Fat Diets on Pigeons Susceptible and Resistant to Atherosclerosis

THOMAS B. CLARKSON and HUGH B. LOFLAND

doi: 10.1161/01.RES.9.1.106

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1961 American Heart Association, Inc. 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/content/9/1/106

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

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

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