The records of the Philadelphia Zoological Garden suggest that social (intraspecific) interaction may be a major factor in the origin and progression of stenosing lesions of the coronary arteries that lead to myocardial infarction in many species of mammals and birds. The present study was designed to explore this possibility. The origin and progress of coronary arterial lesions in cocks and hens have been traced from age 7 to 51 weeks, and rates of progress of the lesions measured quantitatively and related to age, sex, and social situation.

Methods

Chickens from a closed flock of pullorum-free, single-comb, white leghorns of the Hy-line strain were hatched in one lot and brooded as one flock through 7 weeks of age then assigned randomly to cages in one large room with 15 hours of light per day. Ten each of cocks and hens were killed as a sample of brooder stock at 7 weeks of age. The birds were vaccinated and their beaks trimmed at appropriate intervals. Commercial poultry rations were fed ad libitum and water from a deep well was supplied through an automatic system. Cocks weighed 2 to 3 kg and hens 1 to 2 kg at sexual maturity: about 21 weeks of age.

Two-tiered commercial battery cages divided by wooden partitions allowed 2 square ft per bird in all situations except one in which 4 groups of 12 (6 cocks-6 hens) were allowed 8 square ft per bird.

The chickens were studied in the following numbers and situations: a) 40 each of cocks and hens caged separately, b) 64 cock-hen pairs, c) 24 groups of 3 cocks-3 hens, and 10 groups of 6 cocks-6 hens, d) 4 groups of 5 cocks-1 hen, and 4 groups of 10 cocks-2 hens, e) 4 groups each of 1 cock-5 hens, and 2 cocks-10 hens, and f) 4 groups each of 6 cocks, 12 cocks, 6 hens, and 12 hens.

Seven hundred and sixty chickens were started in these six situations, but losses in situations d, e, and f and in two of the 6 cock-6 hen groups, c, were so great that the mean heart scores of these groups have been omitted from the present analyses.

Groups, pairs, and separates were killed for study at weeks 17, 19, 20, 21, 23, 27, 35, and 51 according to the following schedule: 4 to 8 each of separated cocks and hens, 4 cock-hen pairs, and 3 groups of 3 cocks-3 hens. At 23, 27, and 35 weeks two, two, and four of the 6 cock-6 hen groups were also killed.

The birds were killed by decapitation. Body weight was recorded immediately before death and tissue samples immersed in 10% buffered neutral formalin within 10 minutes after death. Testes and adrenals were weighed after fixation. Hearts, kidneys, spleens, livers, and gonads of birds that died were also preserved for study, usually within 10 hours after death.

Segments of hearts, kidneys, adrenals, testes, and spleens were embedded in paraffin, sectioned at 5 micra and stained by hematoxylin and eosin. Sections of hearts and kidneys were also stained for elastic fibers by Verhoeff or Weigert-van Gieson, sections of hearts and kidneys by Lillie's allochrome, and sections of selected hearts by oil red O.

The sections of hearts were cut from a block of tissue taken vertical to the long axis about midway between base and apex to include the walls of both ventricles. The severity of coronary arterial disease in each heart was determined from a section stained for elastic fibers. In these sections arteries greater in luminal diameter than 10 to 15 micra were counted and all examples of intimal thickening graded on a scale 0 to 5; i.e., no intimal thickening to occlusion (grade 1 = less than 25% obstructed, grade 2 = 25 to 50%, grade 3 = 50 to 75% and grade 4 = more than 75%). Then the number of arteries in each grade was multiplied by that grade, the products...
added, and the sum divided by the total number of arteries in the section; e.g., \(1 \times 15 + 2 \times 8 + 3 \times 6 / 56 = 0.87\). For convenience in calculation, 1 was added to this quotient to give the heart score, which in this example was 1.87. Geometric models show that these values are not biased by planes of section.

This is a method of sampling in which results are reproducible and allow statistical treatment. They are, therefore, valid measures of the extent and severity of coronary lesions and a means of relating the disease to experimental situations.

Descriptions of coronary lesions in this series were based upon examination of routine heart sections supplemented by serial sections of specimens from birds killed at increasing ages and selected to study particular lesions. These more detailed examinations were done mainly to determine the extent of lesions in arteries of different sizes and rarely extended through more than 200 micra of tissue. The serial sections demonstrated that appearances of the lesions change from place to place, even in short lengths of arteries.

**Results**

**ORIGIN AND PROGRESS OF CORONARY LESIONS**

Reorientation and proliferation of small groups of cells, apparently smooth muscle cells within the tunica media, were the earliest recognized changes in the coronary arteries of this series. These changes were found in birds killed at week 7, and in general, the size of individual lesions and the number of affected arteries increased with age. All levels of the coronary arterial system from the larger intramural arteries to deeper branches less than 20 micra in luminal diameter were found to be affected by week 20. Lesions in birds of 27 weeks or younger were more frequent in arteries of 40 to 80 micra in luminal diameter. However, the distribution of lesions was not highly regular.

As a rule, focal reorientation and proliferation of cells within the tunica media were closely associated with focal degeneration and disappearance of cells within this layer so that irregular thickening of the arterial wall was associated often with irregularly distributed thinning of the media. Proliferating and degenerating foci that had formed adjacent to the internal elastic membrane were regularly accompanied by fragmentation, disappearance or duplication of this membrane and by extension of the medial cells to form eccentric or irregularly concentric layers beneath the endothelium to thicken arterial walls and to reduce luminal diameters. Overlying endothelial cells often seemed increased in numbers, with nuclei and cell-bodies projecting into the lumen (figs. 1, 2, and 3).

Cells within the medial foci with which this lesion originated, as well as cells in the masses that formed beneath the endothelium, sometimes contained small vacuoles or were separated by irregular spaces. Lipids were rarely demonstrated in these vacuoles and spaces, but these arteries always contained increased amounts of periodic acid Schiff (PAS) positive materials which seemed to be more abundant in the intercellular spaces. Serial sections indicated that numbers of these proliferative and degenerative foci

**FIGURE 1**

Coronary artery about 250 micra luminal diameter from a paired 8, 17 weeks. Lumen to right; internal elastic membrane fractured and duplicated; medial cells form localized mass that projects into lumen. All photomicrographs from sections stained for elastic fibers.

*Circulation Research, Vol. XVII, November 1965*
CORONARY ARTERIAL LESIONS IN CHICKENS

405

CORONARY ARTERY, LUMINAL DIAMETER ROUGHLY 30x60 MICRA, 9, BROODER SAMPLE, 7 WEEKS, FOCAL DEGENERATION AND PROLIFERATION OF MEDIAL CELLS WHICH PROJECT BENEATH ENDOTHELIUM THROUGH BROKEN ELASTIC MEMBRANE.

FIGURE 2

Coronary artery, luminal diameter roughly 30x60 micra, 9, brooder sample, 7 weeks, focal degeneration and proliferation of medial cells which project beneath endothelium through broken elastic membrane.

developed apparently more or less simultaneously in relatively short segments of arteries while immediately contiguous segments appeared unchanged. Further, appearances of adjacent lesions were not uniform. Irregularly arranged concentric intimal thickening might be associated with multiple breaks in the internal elastic membrane with or without obvious foci that disrupted the tunica media. Many examples of complete segmental necrosis of the media were found. In some of these, the internal elastic membrane could not be demonstrated. In others, this membrane was in close contact with collagen fibers of the adventitia (figs. 2, 3, and 4).

The stages of coronary arterial disease illustrated in figures 1, 2, and 3 were rarely found in birds killed during and after week 27, but apparent rates of progress ranged widely. In some instances lesions found in birds at weeks 17 and 20 were more advanced or, at least, had reduced arterial luminal diameters to a greater degree than lesions in birds of weeks 27 and 35. Clearly then, we must complete this description of microscopic appearances without close reference to time sequences, but, of course, recognize that changes found in arteries of large, intermediate, or small size probably are essentially similar, appearances being modified mainly by arterial size. For example, in birds killed during weeks 35 and 51, lesions might be found at any and all levels of the coronary arterial system. However, in arteries of any one size the frequency of lesions, as well as the extent to which luminal diameters were restricted, ranged widely.

In arteries of 10 to 15 micra luminal diameter or smaller, in which an internal elastic membrane was not usually demonstrable, the
common change was a highly uniform thickening of the muscle coat with focal loss of nuclei and hyalinization. This change often involved extensive segments of the arterial system as seen in serial sections and sometimes extended into terminal arterioles.

Lesions of larger intramural arteries, less than 100 micra luminal diameter, could be interpreted usually as extensions of lesions illustrated in figures 1, 2, and 3. In these more extensive lesions the internal elastic membrane was reduced commonly to small scattered fragments located deep in the wall, which suggested rapid loss of cells from the media and rapid ingrowths of cells beneath the endothelium with scanty collagen but without elastic fiber formation (figs. 5 and 6).

A majority of these lesions were solid masses of elongate cells without definite alignment to the axis of the artery. Occasional nuclei were pycnotic and the whole cell mass interlaced with delicate collagen fibers (fig. 5). Less frequently the masses that narrowed the lumen were divided by irregular spaces. These lesions contained relatively coarse strands of collagen and abundant PAS positive materials (fig. 6).

In arteries with luminal diameters greater than 100 micra focal intimal thickening developed apparently in the manner described and illustrated for smaller arteries. Here, however, cells of the thickened intima often contained vacuoles in greater numbers in which lipid was rarely demonstrated. Cells of the media were also often vacuolated and, at times, formed into small nodular masses either deep within the wall or adjacent to the
adventitial collagen. These distorted arterial walls seemed to contain increased numbers of delicate collagen fibers, and always contained increased amounts of PAS positive materials (fig. 7).

In approximately 10% of the birds killed during weeks 35 and 51 the walls of the larger intramural arteries that contained lesions were more or less intensely infiltrated by plasma cells, lymphocytes, and monocytes. These infiltrating cells were most abundant in the thickened intimal tissues and between the collagen fibers of the adventitia. In the media they tended to form small aggregations in spaces from which muscle cells seemed to have disappeared. Many of these cells especially in the media were vacuolated, but lipids were demonstrated rarely. These arteries also were heavily PAS positive (fig. 8).

Serial sections demonstrated that, as a rule, less than half of the damaged segment was infiltrated by plasma cells, lymphocytes and monocytes. Thrombosis was not associated with this change, which is interpreted as secondary to the development of the arterial lesions.

CORONARY LESIONS AND MYOCARDIAL INFARCTION

Relatively small foci of myocardial necrosis and fibrosis were found in sections of the hearts of one cock from each of two 10 cock-2 hen groups that were killed during week 35. One separated cock, also killed during week 35, had developed a small focus of infarction. Two other examples of myocardial infarction in cocks were found at 51 weeks, one from a 3 cock-3 hen group and one from a pair.
Coronary artery, luminal diameter originally about 400 micra, 3 cock-3 hen group, z., rank 3, 35 weeks. Lesion characteristic of larger intramural arteries, but with localized infiltration by leucocytes and monocytes, media irregularly thickened on left and thinned on right, internal elastic membrane fragmented, intima and media contain abundant PAS positive material.

Heart scores for each of these birds were greater than the means for their age.

**RATES OF DEVELOPMENT BY SEX AND AGE**

The coronary arterial lesions that have been described from this series of chickens had begun developing in 8 of 10 hens and in all of 10 cocks that were examined at age 7 weeks. One each of 17 hens killed during weeks 17, 19 and 20 had no lesions, but they were found in all other birds. Thus, the lesions of coronary arterial disease were initiated in both cocks and hens before 7 weeks of age, or 10 to 12 weeks before sexual maturity. Their development generally was more rapid in males, a difference that persisted for the period of this experiment (figs. 9 and 10).

Figure 9 shows the mean heart scores of all cocks and hens killed for study at the intervals stated. Linear regressions of heart scores on age superimposed on these point values illustrate the influence of sex upon the rate of progress of the lesions. The slopes of these regressions differ significantly ($P < 0.05$).

The heart scores of birds killed at age 35 weeks illustrate further an influence of sex on the progress of the coronary lesions (fig. 10). This figure was constructed from the heart scores of 169 cocks and 150 hens and emphasizes normal distributions. Standard deviations are nearly the same for these distributions, but means differ significantly ($P < 0.001$). Heart scores for the cocks were distributed normally about the mean while scores for hens were skewed positively ($P < 0.01$).

**RATES OF DEVELOPMENT—SOCIAL SITUATIONS**

The influence of sex related factors has been emphasized by the analyses shown in figures 9 and 10 without reference to the social situations into which birds had been assigned. The relation of social situations to heart scores in this series is illustrated by figure 11 and table 1.

The analyses given in figure 11 and table 1 are limited to birds in three social situations: a) cocks and hens caged separately, b) cocks and hens of pairs, and c) groups of 3 cocks-3 hens, and of 6 cocks-6 hens. Regression equations of heart score on age were calculated by the method of least squares for birds in each social situation. Group means, i.e., average heart scores for cocks or hens within a group, rather than individual values were used for grouped birds because heart scores within each group were not necessarily distributed normally. In this case the "central limit theorem" was applied since the group means were distributed normally. This treatment also recognizes the group effect or interaction, since a bird cannot be in more than one cage at one time. Degrees of freedom are correspondingly reduced for these regression equations. In examining figure 11
CORONARY ARTERIAL LESIONS IN CHICKENS

Mean heart scores of $\delta\delta$ and $\Omega\Omega$ from all social situations killed for study 7 through 51 weeks. Slopes of linear regressions of heart scores on age diverge significantly ($P < 0.05$).

Distribution of heart scores of $\delta\delta$ and $\Omega\Omega$ at 35 weeks, standard deviation of distributions approximately equal, means differ ($P < 0.001$), $\delta\delta$ scores distributed normally about mean, $\Omega\Omega$ scores skewed positively ($P < 0.01$).

Circulation Research, Vol. XVII, November 1963
it will also be recognized that heart scores for the 10 cocks and 10 hens examined as a sample of brooder stock at 7 weeks were used to calculate each regression.

The slope of the linear regressions on age (fig. 11) indicates that coronary arterial lesions developed most rapidly in cocks that were caged separately and least rapidly in the separately caged hens. Values for males and females of pairs and of groups are not

### TABLE 1

<table>
<thead>
<tr>
<th>Social situation</th>
<th>Numbers</th>
<th>Regression equation: ( y = ax + b )</th>
<th>Standard error of slope ( b )</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cocks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1(\delta) / cage (a)</td>
<td>39</td>
<td>( y = 0.88 + 0.038x )</td>
<td>0.0049</td>
<td></td>
</tr>
<tr>
<td>6(\delta) : 6(\Omega) (c)</td>
<td>8</td>
<td>( y = 0.95 + 0.024x )</td>
<td>0.0020</td>
<td></td>
</tr>
<tr>
<td>1(\delta) : 1(\Omega) (b)</td>
<td>63</td>
<td>( y = 1.14 + 0.019x )</td>
<td>0.0021</td>
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</tr>
<tr>
<td>3(\delta) : 3(\Omega) (c)</td>
<td>24</td>
<td>( y = 1.00 + 0.019x )</td>
<td>0.0014</td>
<td></td>
</tr>
<tr>
<td><strong>Hens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3(\delta) : 3(\Omega) (c)</td>
<td>24</td>
<td>( y = 0.92 + 0.015x )</td>
<td>0.0013</td>
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</tr>
<tr>
<td>6(\delta) : 6(\Omega) (c)</td>
<td>8</td>
<td>( y = 0.98 + 0.016x )</td>
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<td></td>
</tr>
<tr>
<td>1(\delta) : 1(\Omega) (b)</td>
<td>64</td>
<td>( y = 1.06 + 0.012x )</td>
<td>0.0020</td>
<td></td>
</tr>
<tr>
<td>1(\varphi) / cage (a)</td>
<td>28</td>
<td>( y = 1.08 + 0.009x )</td>
<td>0.0041</td>
<td></td>
</tr>
</tbody>
</table>

*Regressions joined by the same vertical line have slopes that do not differ statistically. Slopes of regressions not so joined differ significantly (\(P < 0.05\)).

[Figure 11](#)

*Linear regressions of heart scores on age for \(\delta\) and \(\Omega\): a) caged separately, b) paired, or c) grouped (see table 1).
so widely separated. These differences and similarities are expressed mathematically in table 1.

Table 1 identifies each regression equation with the appropriate social situation as designated in the section on Methods (a), (b), (c), and shows that the rate of development of coronary arterial lesions was significantly higher ($P < 0.05$) in separately caged cocks than in cocks of either pairs or groups. This analysis also shows that the rates at which the lesions developed in females of both types of group was not significantly less rapid than the rate for cocks of the 3 cock-3 hen groups. Thus, rates of progress of the lesions may depend both on sex and the social situation.

The values given in table 1 also indicate that group size (6 vs. 12) was a factor in the rate at which coronary lesions progressed. However, heart scores of cocks and hens ranged widely from group to group without close relation to age (35 vs. 51 weeks) or group size (6 vs. 12). It has been interesting therefore to correct mean heart scores of grouped cocks and hens to age 35 weeks according to values given in figure 9 and to determine their coefficient of correlation. This analysis has shown that high mean heart scores for cocks were more often associated with high scores for hens of the group. Similarly, low scores for cocks were more often associated with corresponding low scores for hens. The coefficient of correlation for this association is 0.38 ($P < 0.01$). This contrasts to the lack of association between heart scores of paired cocks and hens for which the correlation coefficient is 0.06 ($P > 0.10$).

**Discussion**

The coronary lesions found in this series correspond closely in location and other characteristics to coronary lesions that have developed in birds and mammals of this and other zoological gardens. These lesions also closely correspond to the common coronary arterial lesions of domesticated animals, and to lesions of the intramural coronary arteries of mankind. Furthermore, they originate at relatively early ages and begin with focal necrosis and proliferation of smooth muscle cells of the arterial wall, which is in keeping with observations on other species including man.

Differences between coronary lesions in these chickens and the more common lesions of other birds and mammals are: a) greater cellularity, b) scanty collagen formation, and c) more obvious foci of smooth muscle degeneration and proliferation. Experience with a considerable series of zoo birds and mammals of all ages and with hens culled from an egg production flock because of non-productivity at ages 26 to 30 months suggests that these differences reflect rates at which lesions developed. The coronary lesions of young birds and mammals of this zoo characteristically are more cellular than lesions of equal size in older specimens. As a rule, the coronary lesions of mammals contain elastic fibers in greater numbers and with less collagenous material than do the lesions of birds.

This and related studies also suggest an explanation for an earlier claim that intramural coronary disease of chickens is part of the response to the leucosis virus complex. Leucosis was a relatively common cause of death of grouped birds. The increased adrenal cortical activity that accompanied grouping could reduce resistance and activate a latent virus complex. Coronary disease and leucosis therefore may be induced in some animals by the same social situation, but probably not through the same mechanism. As independent phenomena in the same population, their association should have been random which it was in this series.

Since 1945, efforts to assemble and maintain breeding stocks of mammals and birds in this zoological garden have been followed by increased frequency of stenosing lesions of the intramural coronary arteries and myocardial infarction. Diets have been constant since 1935 and other maintenance factors have undergone little change.

Behavior patterns that include continuous male-female association in pairs and groups...
are rare among wild animals. Forced continuous association of male with female in this zoo has been accompanied by interrupted pregnancies, failure in lactation, premature involution of the gonads, and hypertrophy of the adrenals. These changes are also characteristic of confined freely growing rodent populations, and are responses to levels of social interaction in these populations. Hence the increased frequency of coronary lesions and myocardial infarction in the animals of this zoo also have been attributed to the increased levels of social interaction since 1945.

In the present study the social hierarchy and level of interaction in each group were determined by direct observations guided by accepted criteria. The frequency of competitive contacts (social interaction) in the 6 cock-6 hen groups ranged from 1.4 to 7.3 times higher than in the 3 cock-3 hen groups. Involvement of the gonads and hypertrophy of the adrenals were found regularly in the subordinate birds of these and other groups.

At this time it is not profitable to attempt to relate the rates of development of coronary lesions to dominance-subordinance relationships within groups or to levels of group interaction. This will be done when study of material from subsequent experiments is completed, but it may be noted here that heart scores of grouped birds were not always correlated with social rank. Furthermore, heart scores of grouped hens were sometimes higher than scores of cocks of the same groups.

Earlier observations on zoo animals also had led us to suggest a relation between responses of the adrenals and gonads to group interaction and the development of coronary arterial disease. In the present study however, gonads of separately caged cocks were significantly heavier and adrenals significantly smaller (P < 0.05) than these organs of grouped cocks. Thus, reports of an association between adrenal hyperplasia and coronary disease may reflect a spurious correlation.

Among zoo animals, rapidly developing coronary disease in separately caged animals has been associated with changes or disturbances in behavior patterns in response to social interaction among animals in nearby cages. We suggest, therefore, that responses to visual and auditory stimuli from nearby pairs and groups may explain the more rapid development of coronary disease in separately caged cocks.

In spite of its more rapid progress, coronary disease in separately caged cocks was not accompanied by ischemic necrosis of the myocardium. This occurred only in the presence of females, which corresponds to our other observations on coronary disease and myocardial infarction in chickens. Responses at a distance to the stimuli of social interaction may induce rapid development of coronary lesions. Within the period of this experiment, however, the added stimulus of contact with hens was necessary to induce ischemic necrosis of the myocardium.

Summary

Single-comb, Hy-line white leghorns were hatched in one lot, brooded in one flock through 7 weeks, then assigned randomly to cages in one large room with 15 hours of light per day. The birds were studied in the following situations: a) separately caged cocks and hens, b) paired cocks and hens, and c) 3 cock-3 hen groups and 6 cock-6 hen groups. Basic space allowance was 2 square ft per bird which was increased to 8 square ft for some of the larger groups. Commercial rations and water were supplied ad libitum. Stenosing lesions of the intramural coronary arteries began development in cocks and hens before age 7 weeks (10 to 12 weeks before sexual maturity) and progressed with age. Progress, as measured by heart scores, was not influenced by space allowance but was related to social situation. The rate for separately caged cocks was significantly higher than for other birds. Cocks of the larger groups and cocks of the pairs were next in order, while heart scores for cocks of the smaller groups were third but not significantly higher than for grouped hens. Heart scores of grouped hens sometimes exceeded heart scores for cocks of corresponding groups.
Circulation Research, Vol. XVII, November 1965

CORONARY ARTERIAL LESIONS IN CHICKENS

High mean heart scores for grouped cocks correlated with high mean heart scores for hens of their groups, and low heart scores for grouped cocks with low scores for hens of their groups.

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HERBERT L. RATCLIFFE and ROBERT L. SNYDER

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