Group Isolation and Atherosclerosis in Cholesterol-Fed Cockerels

By Ruth Pick, M.D., Louis N. Katz, M.D., Dolores Century, B.S., and Philip J. Johnson

It has been suggested that emotional stresses may be involved in the rate of development of atherosclerosis, may act to precipitate an acute arterial occlusion in the presence of atherosclerotic vascular disease, or, in the case of muscular organs like the heart, may serve to enhance the rate of activity of the organ beyond the capacity of the vasculature to prevent acute ischemia, thereby triggering an ischemic episode.

Clinical studies on the role of psychological stresses in atherosclerosis are very difficult to carry out. The main reasons for these difficulties lie in the lack of a precise measure by which to characterize psychological stresses, and to adapt such stress evaluation to the reaction of a given individual.

Studies on animals from the Philadelphia Zoological Garden indicate that over the past 50 years there was an increase in the occurrence of spontaneous arteriosclerosis in mammals and birds. The first increase was noted to occur following 1936 after drastic dietary changes. Since then, diet has been kept constant. Nevertheless, a second increase occurred with a change in distribution of the lesions. This second increase was attributed to changes in the environment of the animals, namely an increase in cage population, noise, and frequency of contact with man.

Experimental results from the animal laboratory on the influence of stress on atherosclerosis are fragmentary and contradictory. Cold-stress has been reported to produce coronary artery changes in rats. A similar effect has been reported following prolonged swimming in cold water. On the other hand, no difference in the degree of coronary atherosclerosis is said to exist between aggressive and passive chicks on an atherogenic diet.

No differences in the degree of cholesterolemia or coronary and aortic atherogenesis in cholesterol-fed cockerels were found when conditioned anxiety was produced by repeated electrical shocks. Chronic hypothalamic stimulation of cholesterol-fed rabbits was reported to increase hypercholesteremia and atherogenesis. Others have demonstrated a decrease in atherogenesis in cholesterol-fed rabbits given central nervous system depressing drugs, and an increase in atherogenesis when stimulating drugs were given.

The present report deals with an attempt to evaluate some environmental influences on the development and regression of diet-induced aorta and coronary atherosclerosis in chicks. This animal species lends itself readily to such experiments, since it has been shown that chickens establish a strict social system, the so-called "pecking order," within weeks, or at least within several days, after being placed in a flock or a cage. Also, birds are naturally gregarious, normally living in large flocks. This makes it possible to study: 1) the effect of isolation of small groups of animals in a quiet environment and 2) the effects of disturbing the social system with consequent fighting among the chickens for new positions of rank.

Methods

Five series (S55, 59, 62, 65, 68) of experiments were carried out involving 383 Hy-Line cock-
ERELS. All were received in the laboratory when one day old, were reared in a battery brooder until they were five weeks of age, when they were transferred to cages of four tiers with ten birds per tier. Until the experiments started the birds were maintained on regular chick starter mash. The atherogenic diet during the experimental period consisted of regular mash supplemented with 1% cholesterol and 5% cottonseed oil by weight to which sufficient sucrose was added to reduce the protein content in the diet to 15% from the normal 20%. The experimental period lasted from five to eight weeks. The birds were from nine to thirteen weeks old at the start of the experiment. This was the experimental design utilized in studying induction of atherosclerosis.

When regression of atherosclerosis was under study the atherogenic diet was given for only five weeks after which regular mash was substituted for a period of three weeks (1 CO—5 weeks → RM 3 weeks). This procedure leads to regression of coronary lesions and lowering of blood cholesterol. The procedure of moving birds, of handling them, and of placing them in the isolated rooms was carried out in the regression studies only at the time when the birds were placed on regular mash.

Group isolation of birds was studied by placing 40 birds (1 CO—Group isolated) in a tiered single cage (with ten birds in each of the four tiers) in a separate room which was kept as quiet as possible, being removed not only from the turmoil created by the large number of chicks in the regular chicken room but also that resulting from the usual daily repeated contact with personnel working in the regular chicken room. The room with the isolated birds was entered only three times weekly by a single person in order to clean, feed, and water the animals. Even these visits by personnel were organized so as to cause as little disturbance as possible. The controls for this experiment (1 CO—Control) were kept in the large chicken room and handled similarly to the undisturbed control group as described below.

In another group of experiments, disturbances in the pecking order were created by randomly altering the population of several cages of birds two or three times a week by moving some of the birds from one cage to another (1 CO Shifting population). As a control some cages of
birds housed in the same room were disturbed by handling, but without shifting the population, every time the birds in the experimental cages were rearranged (1 CO Disturbed controls). A second control consisted of birds in cages, in the same room as those mentioned above, which were not disturbed except by cleaning the tray under each tier and changing the feed and water (1 CO Undisturbed controls).

Birds were weighed at the beginning and end of an experiment and weekly food intakes were determined. At sacrifice, comb indices were measured and testes weights recorded.

Plasma cholesterol was determined by the method of Sperry and Webb. Aortic atherosclerosis was evaluated according to the established methods of this department, using a grade from 0 (no lesion) to 4+ (the most severe). The extent of coronary atherosclerosis was determined by taking one frozen section stained with Sudan IV from each of two blocks of each heart and calculating the percentage of atherosclerotic vessels.

Results

The birds subjected to group isolation showed marked changes in atherogenesis (fig. 1). Diet-induced coronary atherosclerosis was significantly enhanced in the birds subjected to group isolation (1 CO Group isolated) as compared to birds in the regular chicken room (1 CO Control) (32.4% vs 21.9%, \( P < 0.001 \)) despite similar plasma cholesterol levels (fig. 1). Regression of previously induced atherosclerosis during the three weeks of regular mash feeding after five weeks on the atherogenic diet was also significantly delayed in birds that were isolated compared to birds in the regular chicken room (the incidence of coronary lesions in the isolated group regressed from 25.7% (1 CO 5 weeks) to only 24.1% (1 CO 5 weeks → RM 3 weeks—Group isolated) while that in the controls regressed to 14.8% (1 CO 5 weeks → RM 3 weeks—Control); \( P < 0.001 \) for the difference between the two groups) (fig. 2).

The isolated birds (1 CO—Group isolated) had smaller weight gain compared to birds in the regular chicken room (1 CO—Control)}
GROUP ISOLATION AND ATHEROSCLEROSIS

TABLE 1

Effects of Group Isolation of Cockerels (Series 62 and 65)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>Food intake g/bird/day</th>
<th>Δ Wt g</th>
<th>Comb index</th>
<th>Testes wt g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CO-Control</td>
<td>58</td>
<td>108</td>
<td>+468</td>
<td>±34</td>
<td>±2.9</td>
</tr>
<tr>
<td>1 CO-Group isolated</td>
<td>38</td>
<td>100</td>
<td>+296</td>
<td>±17</td>
<td>±4.8</td>
</tr>
</tbody>
</table>

P values

Control vs Group isolated — — <0.001 <0.05 <0.001

Table organized as in table 3.

TABLE 2

Effects of Group Isolation in Regression Experiments on Cockerels (Series 65 and 68)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>Food intake g/bird/day</th>
<th>Δ Wt g</th>
<th>Comb index</th>
<th>Testes wt g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1 CO 5 wk</td>
<td>78</td>
<td>101</td>
<td>+556</td>
<td>±18</td>
<td>±3.3</td>
</tr>
<tr>
<td>2. 1 CO 5 wk -&gt; RM 3 wk</td>
<td>56</td>
<td>113</td>
<td>+246</td>
<td>±23</td>
<td>±2.2</td>
</tr>
<tr>
<td>Control</td>
<td>56</td>
<td>111</td>
<td>+94</td>
<td>±19</td>
<td>±2.1</td>
</tr>
<tr>
<td>3. 1 CO 5 wk -&gt; RM 3 wk Group &quot;isolated&quot;</td>
<td>56</td>
<td>111</td>
<td>+94</td>
<td>±19</td>
<td>±2.1</td>
</tr>
</tbody>
</table>

P values

1 vs 2 — — — <0.001 <0.001
1 vs 3 — — — N.S. N.S.
2 vs 3 — — <0.001 <0.001 <0.001

*RM is regular mash.
Table otherwise organized as in table 3.

despite comparable food intakes, suggesting a decreased food utilization (table 1). In addition, their sexual development was retarded. In the regression experiments, isolation during these three weeks (1 CO 5 weeks -> RM 3 weeks—Group isolated) again led to significantly decreased food utilization and sexual development as compared to the controls (1 CO 5 weeks -> RM 3 weeks Control) (table 2), and a smaller decline in blood cholesterol levels (compare 1 CO 5 weeks -> RM 3 weeks—Group isolated with 1 CO 5 weeks -> RM 3 weeks—Control in fig. 2).

In addition, changes in feathering and extreme excitability were noted in isolated birds when they were removed from their isolated environment before sacrifice at the end of the experiment.

While disturbing the pecking order of the cockerels (1 CO Shifting population) resulted in enhanced sexual development, as evidenced by increased comb and testes size, it affected food intake, hypercholesterolemia, and aortic and coronary atherogenesis little, or not at all compared to the controls (1 CO Disturbed Controls and 1 CO Undisturbed Controls) in the regular chicken room (table 3). Table 3 also shows that weight gain was significantly greater in the control groups (see above) than in the group with shifting population. Thus, the stress of fighting to re-establish the social order did not influence the atherogenic response to the diet, even though evidence of masculinity was enhanced.

Discussion

The data of these experiments clearly indicate that certain environmental factors, which might alter the psychological state of cock-
TABLE 3
Effects of Disturbing the Pecking Order in Cockerels on Hypercholesterolemia, Atherogenesis, and Other Parameters (Series 58, 59, 62, and 65)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>Food intake g/bird/day</th>
<th>Δ Wt g††</th>
<th>Comb index‡</th>
<th>Plasma cholesterol mg %</th>
<th>Thoracic aorta lesions Incidence</th>
<th>Thoracic aorta lesions Grade</th>
<th>Coronary lesions Incidence</th>
<th>Coronary lesions % Involvement**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1 CO*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disturbed controls‡‡</td>
<td>60</td>
<td>112</td>
<td>+373</td>
<td>72.4</td>
<td>4.42</td>
<td>1654</td>
<td>100%</td>
<td>1.81</td>
<td>100%</td>
</tr>
<tr>
<td>Undisturbed controls†</td>
<td></td>
<td></td>
<td>± 30§</td>
<td>± 2.8</td>
<td>±0.50</td>
<td>±65</td>
<td>±0.10</td>
<td>∓ 1.3</td>
<td></td>
</tr>
<tr>
<td>2. 1 CO</td>
<td>38</td>
<td>124</td>
<td>+504</td>
<td>72.9</td>
<td>4.22</td>
<td>1446</td>
<td>100%</td>
<td>1.51</td>
<td>100%</td>
</tr>
<tr>
<td>Shifting population↑</td>
<td>77</td>
<td>119</td>
<td>+390</td>
<td>83.8</td>
<td>7.03</td>
<td>1346</td>
<td>100%</td>
<td>1.98</td>
<td>96%</td>
</tr>
<tr>
<td>3. 1 CO</td>
<td></td>
<td></td>
<td>± 16</td>
<td>± 3.0</td>
<td>±0.61</td>
<td>±62</td>
<td>±0.10</td>
<td>∓ 1.3</td>
<td></td>
</tr>
</tbody>
</table>

P values

| 1 vs 2                  |              |                        | <0.005   | N.S.|| | N.S. | N.S. | —     | N.S. | —     | N.S. |
| 1 vs 3                  |              |                        | N.S.     | <0.01   | <0.005 | <0.005 | —     | N.S. | —     | N.S. |
| 2 vs 3                  |              |                        | <0.001   | <0.05   | <0.005 | N.S.   | —     | <0.01 | —     | N.S. |

*1 CO is 1% cholesterol + 5% cottonseed oil in chick starter mash.
†See text.
‡Product of greatest length by height of comb directly over the eye.
§Standard error of the mean.
||N.S. = not significant.
**% of vessels with atheromata.
††Δ wt is weight gain.

*1 CO is 1% cholesterol + 5% cottonseed oil in chick starter mash.
†See text.
‡Product of greatest length by height of comb directly over the eye.
§Standard error of the mean.
||N.S. = not significant.
**% of vessels with atheromata.
††Δ wt is weight gain.
erels, can influence the development and regression of dietary-induced atherogenesis. Such an unnatural situation was created by group isolation of birds, which leads to a reduction in the amount of extraneous stimulation. On the other hand, neither the handling of birds, which augments the amount of their stimulation, nor our method of disturbing the pecking order, which results in obvious sustained fighting among the birds, altered the atherogenic response to the diet.

The mechanism underlying the effect of group isolation on atherosclerosis is not clear. Decreased physical activity in the isolated birds as well as altered pattern of food intake have to be considered. However, the smaller weight gain in these isolated birds despite food intakes comparable with the control group makes physical activity per se an unlikely explanation.

The effect of group isolation in the induction experiments is not dependent upon the blood cholesterol level change since the degree of hypercholesterolemia was similar in the group isolated birds and their controls. The effect of group isolation therefore must be sought either in the nature of the blood lipoproteins or in some direct effect upon the blood vessels. In the regression experiments, however, the results on atherosclerosis regression may have been influenced by the smaller drop in blood cholesterol levels during group isolation.

Even though group isolation involves isolation of 40 birds in a cage in one room and not isolation of each individual bird, this represents a highly unnatural situation for chicks. The effects observed may be due to lack of the usual, ordinary stimulation or to the fact that this unnatural environment creates a stressful chain of events which biologically is more than mere absence of ordinary stimulation. Whether the lack of visual or acoustic stimulation is the decisive factor is currently under study. The data clearly indicate that certain environmental influences can affect the vascular response to an atherogenic diet.

There is a great deal of evidence that individual isolation in many species of animals causes profound behavioral deviations. However, there are no studies, to our knowledge, on group isolation other than the one presented here.

Stress accompanying augmentation of environmental stimulation such as electric shock or disturbing the pecking order, as done in the present study, had no effect—neither retarding nor enhancing atherogenesis. Obviously, stress and stressful situations cannot be merged into a single category nor need they act in the same manner in different species.

Our results show that disturbance in the pecking order is accompanied by an increase in secondary sex characteristics in the birds whereas deprivation of environmental stimulation is associated with a reduction. This suggests that the development of male sexual characteristics is affected by the amount of turmoil and activity created by the environment in which the birds reside. The relation of these environmental changes to endocrine disturbances, weight gain, and atherogenesis and its regression needs to be worked out.

Group isolation of birds augments atherogenesis and retards sexual development, whereas handling of birds and changing the pecking order fails to affect atherogenesis while enhancing sexual development. This suggests either: 1) that augmenting the amount of stimulation in the environment has no effect on atherogenesis, or 2) that augmented environmental stimulation initiates two opposite effects which neutralize each other. Future work is obviously necessary to decide these and other problems posed by the present studies.

**Summary**

Group isolation, a highly unnatural environmental situation in cockerels, increases atherogenesis and decreases regression of previously induced atherosclerosis. This alteration occurs despite the absence of change in the hypercholesterolemic response to the diet in the induction experiments. It is accom-
panied by a reduction in weight gain despite food intake similar to the controls.

Disturbing the social order by altering the pecking order in flocks of cockerels on a cholesterol-oil containing diet does not alter hypercholesterolemia and atherogenesis. However, it leads to increase in sex characteristics. Physical activity, with or without alterations in pecking order, causes a reduced weight gain even when food intakes are not altered.

It is concluded that environmental factors can and do influence atherosclerosis. The types of environmental change which can produce this effect and the exact mechanisms involved require further study.

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References

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