Studies on the Relation of Diet, Cholesterol and Atheroma in Chickens

By John E. Peterson, M.D., and Albert E. Hirst, M.D.

In this study cockerels were fed varying diets supplemented with lard, vegetable oil, and cholesterol. The degree of atherosclerosis was compared in serial autopsies and only the cholesterol-fed group showed a significant difference. During a period of two years there was no apparent change in the degree of atheroma when the diet was supplemented with cottonseed oil or lard. Reversibility of the cholesterol-induced atheroma was also studied in a few chickens, and it appears that the early lesions may undergo considerable regression after cholesterol feeding is stopped. Older lesions, on the other hand, appear to be quite fixed.

The spontaneous development of atherosclerosis in domestic chickens is well recognized and the vascular lesions have been compared closely with atheromatous changes in man. The significance of such lesions, occurring spontaneously and after cholesterol feeding in an omnivorous animal such as the chicken, has been emphasized by Dauber and Katz. Though both rabbits and fowl appeared to develop atheromatosis more readily when the added cholesterol is dissolved in some oil, it has been shown in chickens that cottonseed oil alone will not produce such lesions. While the atheroma-producing effects of various diets have been attributed to some slight cholesterol content, it is now known that in rabbits the feeding of a protein diet free or almost free from cholesterol will result rapidly in vascular changes of the atheromatous type. Atherosclerosis following the injection of colloidal solutions of macromolecular carbohydrates also has been reported by Hueper.

With the above observations in mind, the authors became interested in studying the effects of long term feeding of fat from plant and animal origin. Lard was chosen for the animal fat because of its low cholesterol content. The results of these feeding programs are compared with the findings in chickens fed cholesterol and with those on a standard diet of mash.

Anitschkow and others have reported the regression of atheromatous lesions in the aorta of rabbits. A second phase of this report deals briefly with this aspect.

Procedure

For this study 100 healthy-appearing white Leghorn cockerels were picked from the large stock of a commercial grower. The birds, when selected, were 10 weeks old and of similar size and weight. The 100 were divided into four groups, and each group was kept in a standard cage on wire. Fresh water was always available, and only the diet of each group was different.

The first group of 25 chickens (group A) served as a control and was fed a standard mash* ad libitum. Fresh feed was provided each day. The amount consumed was carefully measured by weighing the residue from the previous day before discarding it and adding fresh mash.

A second group of 25 (group B) was fed similarly except that a vegetable oil (chiefly cottonseed) was added to the standard mash. This feed was prepared freshly each day by thoroughly mixing a pint of oil with five pounds of dry mash—a mixture of approximately 20 per cent volume by weight.

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* Globe Mills Battery Mash: 20% protein, containing ground corn 600, wheat bran 500, soybean meal 300, ground wheat 300, ground milo 300, fish meal 200, condensed fish solubles 200, alfalfa meal 200, wheat middlings 200, meat scraps 100, fortified whey solids 50, lime stone meal 50, iodized salt 15, fortified feeding oil (1000 A-400 D)4, manganese sulfate .375.

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A third group of 25 (group C) was fed in a manner similar to groups A and B except that a pint of lard instead of vegetable oil was added to each five pounds of dry mash.

Feed for the fourth group (group D) was prepared in the same way as for group B except that 2 Gm. of crystalline cholesterol was added for each 100 Gm. of dry mash fed. The cholesterol was first dissolved in the vegetable oil then this solution was thoroughly mixed with the mash.

Results

As shown in table 1, there is little difference in the amount of atheroma found in the first three groups (groups A, B, and C). However, as expected from previous studies, there was severe and extensive atherosclerosis in all the birds kept on the cholesterol ration (group D). A grading system was used for the gross lesions so that “plus 1” signifies no more than a lipoidal streak or a tiny plaque measuring not more than 5 mm. in length—a minimal lesion.

Table 1

<table>
<thead>
<tr>
<th>Autopsy No.</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
<th>7.</th>
<th>8.</th>
<th>9.</th>
<th>10.</th>
<th>11.</th>
<th>12.</th>
<th>13.</th>
<th>14.</th>
<th>15.</th>
<th>16.</th>
<th>17.</th>
<th>18.</th>
<th>19.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control mash</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>B. Mash plus vegetable oil</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>C. Mash plus lard</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>D. Mash plus vegetable oil &amp; cholesterol</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<td>+</td>
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<td>0</td>
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<td>0</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>+++</td>
</tr>
</tbody>
</table>

Code: +---Minimal atherosclerosis
++++---Moderate
++----Severe
C---Calcification

After 80 days on this diet, a bird from each group was sacrificed by bleeding and the organs subjected to gross and microscopic examination. Similar studies were made in the remaining birds, first at intervals of two weeks, and later at longer intervals.

As this report deals primarily with the vascular changes found, it should be noted that at autopsy the heart and aorta were promptly removed and examined for gross signs of atheromatous disease. The structures were then fixed in 10 per cent formalin and sections were made from the heart, the coronary arteries, and the abdominal aorta. Additional sections were made wherever gross lesions were found. “Plus 2” denotes an atheromatous plaque but not exceeding 15 mm. in length. A grade of “plus 3” is applied to severe atherosclerosis with multiple plaques. “C” indicates calcification.

It is noteworthy that all the lesions rated as “plus 1” or “plus 2” were found in the descending aorta just above the bifurcation. The relation between “plus 2” and “plus 3” hardly indicates the striking difference found in the cholesterol-fed birds. All these chickens in group D showed severe atherosclerosis with multiple plaques about the coronary ostia, throughout the aorta, and extending into the
larger branches. In some areas these were calcified.

It is interesting that the cholesterol-fed birds were much more irritable and combative than their fellows. They ate less food and were smaller. Dauber and Katz have shown, however, that under-feeding alone will not cause such atheromatous changes. The fowl on a high fat diet (both vegetable oil and lard) had a poorer appearance than the controls. Their feathers were greasy and their combs were less red. They were a little more irritable, especially in hot weather; but the gross and microscopic appearance of their organs was not significantly different.

** Attempted Reversal of Lesions

After four and one-half months on this diet, well-established atherosclerosis was found uniformly in the autopsied birds that had been fed cholesterol. Three of the remaining birds were then removed from group D. They were banded and placed in a pen with a group of cockerels that were being underfed by allowing 70 per cent of the amount of standard mash fed to the control group. Their general appearance improved rapidly, and within a few weeks they could not be distinguished from others in the pen except for their identifying leg bands. Four months later one of these birds was examined, and the degree of atherosclerosis is noted in table 2 (group F).

Only some yellowish discoloration and a lipoidal streak were found in the descending aorta. No other gross evidences of atherosclerosis were found, and the heart and aorta appeared similar to those of the controls. Subsequent study of the other 2 of these 3 chickens revealed no more than minimal atherosclerosis in their aortas.

**Table 2**

<table>
<thead>
<tr>
<th>Autopsy No.</th>
<th>Date</th>
<th>E. Mash 30% underfed</th>
<th>F. Off cholesterol on 23 Mar. '48, underfed since then</th>
<th>G. Off cholesterol on 6 Aug. '48, normal mash since then</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>22 July</td>
<td>+</td>
<td>+</td>
<td>++, C</td>
</tr>
<tr>
<td>14</td>
<td>5 Aug. 1948</td>
<td>0</td>
<td>+</td>
<td>++, C</td>
</tr>
<tr>
<td>15</td>
<td>23 Aug. 1948</td>
<td>0</td>
<td>+</td>
<td>++, C</td>
</tr>
<tr>
<td>16</td>
<td>4 Sept. 1948</td>
<td>0</td>
<td>+</td>
<td>++, C</td>
</tr>
<tr>
<td>17</td>
<td>8 Oct. 1948</td>
<td>0</td>
<td>+</td>
<td>++, C</td>
</tr>
<tr>
<td>18</td>
<td>11 Nov. 1948</td>
<td>0</td>
<td>+</td>
<td>++, C</td>
</tr>
<tr>
<td>19</td>
<td>19 Jan. 1950</td>
<td>0</td>
<td>+</td>
<td>++, C</td>
</tr>
</tbody>
</table>

**Code:**
- + — Minimal Atherosclerosis
- ++ — Moderate
- +++ — Severe
- C — Calcification

After nine months of cholesterol feeding, 3 more birds were removed from group D. These were banded and fed with the controls in group A. The general appearance of these 3 improved, though not as quickly or to the extent that it had in the preceding 3 chickens that had been removed four and one-half months earlier from the cholesterol-fed group.

Three months after withdrawing cholesterol all 3 of these birds were autopsied and the degree of atherosclerosis is shown in table 2 (group G). While the lesions may have been a little less severe than in the birds autopsied in the course of cholesterol feeding, there was
extensive atherosclerosis throughout the aorta with several calcific plaques. This appeared in striking contrast to the minimal changes found in the birds that had been earlier withdrawn from cholesterol feeding.

**Discussion**

Until a larger variety of fats has been tried, it would be unreasonable to conclude that no difference in the atherosclerosis of chickens follows the feeding of animal or vegetable fats. There appears, however, to be no significant difference in the amount of atheroma occurring in cockerels fed comparable amounts of cottonseed oil and lard. With the addition of crystalline cholesterol to the cottonseed oil, there is rapid development of severe atheroma and a considerable increase in the total lipids, neutral fat, and fatty acids in the liver.7

Whether the apparent difference in the reversibility of the cholesterol-induced atheroma of chickens is due to the age of the bird, or to underfeeding, or to the lesser exposure to cholesterol, cannot be determined from this study. In comparing a small group of similarly underfed birds with controls on a normal diet, there appeared, however, to be no significant difference in the amount of atherosclerosis found. While there was some increase in spontaneous atherosclerosis in the older birds (up to two and a half years), this difference was slight compared to the birds fed cholesterol. It seems more likely then, that reversibility is related to the duration and total amount of cholesterol feeding than to these other factors. This view corresponds with Anitschkow’s interpretation of the regressive changes occurring in rabbits.6

**Summary**

One hundred cockerels were fed various diets, supplemented with lard, vegetable oil, and vegetable oil with added cholesterol. The degree of atherosclerosis in these birds was compared at serial autopsies, and only the cholesterol-fed group showed a significant difference. In this latter group severe atherosclerosis developed rapidly and there was a considerable increase in the lipid content of the liver.

Observations in a small group of chickens suggest that if the feeding of excessive amounts of cholesterol is stopped in time, there may be some reversal of the atheromatous lesions. Later the regression of such lesions may be much less complete.

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