Hypercholesteremia and Atheromatosis in Chicks on a Restricted Diet Containing Cholesterol

By S. Rodbard, Ph.D., C. Bolene, M.S., and L. N. Katz, M.D.

Restriction of dietary intake even to the point of emaciation gave no protection against atheromatosis or hypercholesteremia in chicks on a diet supplemented with cholesterol. These results show that there is no necessary relationship between the amount of body fat and atherosclerosis.

Excess caloric intake and obesity have long been implicated in the development of atherosclerosis. This concept has been fostered by the common findings of an increased incidence of aortic and coronary atheromatosis in necropsies on overweight individuals.1-4 Recent experiences arising in the backwash of the two great wars have been interpreted as strengthening this belief since the incidence of atherosclerosis was low in famine areas and concentration camps but continued high in well fed troops.2 The apparent freedom of the arterial tree from atheromatosis in starvation is illustrated by the fact that only a single reference to arterial pathology is given in the extensive review on human starvation by Keys and his co-workers.5 However, there is considerable evidence which indicates that atherosclerosis may be closely associated with the composition of the diet rather than its amount. Thus atherosclerosis is uncommon among well fed Chinese6 and Okinawans.7 The presumed correlation between obesity and atheromatosis may therefore be challenged.

The finding that in some populations lavish caloric intake is correlated with atherosclerosis may not be dependent upon overfeeding as such. Instead it may be a function of a superfluity of some specific constituents of the diet, which incidentally are taken in excess by individuals on a luxus food intake. Support for this thesis has recently come in statistical analyses of the mortality experiences in the Scandinavian countries.8, 9 In Denmark and in Norway a marked drop in mortality from circulatory diseases occurred during the German occupation. This was particularly notable in urban areas; it existed to a lesser degree in the rural districts where cholesterol-rich foods were still available in limited quantities.

The increasing evidence for the atherogenic role of cholesterol makes it necessary to assay the relative effects of excess calories and excess cholesterol intake in the production of arterial lesions.

A subsidiary problem concerns the possible utilization of excess dietary cholesterol for nutritional purposes in conditions of caloric privation. If this occurred, it might be expected that starved animals on such a cholesterol enriched diet would utilize the excess cholesterol and thus reduce the tendency to atheromatosis. It was therefore decided to study the tendency to hypercholesteremia and atheromatosis in animals on a restricted food intake high in cholesterol.

Methods and Results

To study these questions, 40 chicks, divided into three groups, were given a restricted diet enriched with either 8 per cent, 2 per cent or 0.25 per cent cholesterol. Control groups of chicks totaling 10 birds, received the same

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During the course of this study, one of us (C. B.) was Deborah V. Dauber Memorial Research Assistant.
diets ad libitum. The amount of cholesterol mash given to the animals on the restricted diet was about 60 per cent of that taken by the control groups. Six chicks received normal mash diets.

All these animals were carried until incidental death in the course of the experiment or until they were sacrificed at the end of 5, 10 or 15 weeks on the special diet.

The average plasma cholesterol increased from about 100 mg. per 100 cc. at the beginning of the experiment to 300 mg. per 100 cc. in one week of feeding. This approximate level was maintained, being 450 at five weeks, 400 at 10 weeks and 300 at 15 weeks.

One of 7 animals autopsied during the fifth week of feeding showed mild (grade 1) thoracic atheromatosis. Four of 9 animals autopsied at the tenth week had lesions graded as mild to severe (\(\frac{1}{2}\) to 3\(\frac{1}{2}\)). Four of the remaining 6 animals which were autopsied at the end of the fifteenth week had lesions varying from \(\frac{1}{2}\) to 1.

Three of these 6 animals were found at autopsy to be females. It was in 2 of these that no lesions were found. In the third, mild thoracic atherosclerosis graded as \(\frac{1}{2}\) was observed.

Despite the restricted food intake, and the evident emaciation of the animals, atheromatosis was seen in 9 of 22 animals. This showed that atherogenesis may occur despite failure to gain weight at a normal rate.

**Table 1.**—Plasma Cholesterol Levels and Atheromatosis of Chicks on Full and Restricted Diets

<table>
<thead>
<tr>
<th>Weeks on diet</th>
<th>No. of chicks</th>
<th>Percentage of thoracic lesions</th>
<th>Average grade of thoracic lesions. Birds with lesions</th>
<th>Index</th>
<th>Plasma Cholesterol level mg. per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\frac{1}{2}) CO*</td>
<td>25</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>(\frac{1}{2}) CO restricted diet</td>
<td>18-24</td>
<td>6</td>
<td>50</td>
<td>1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>2 C†</td>
<td>15</td>
<td>5</td>
<td>80</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>2 C restricted diet</td>
<td>15</td>
<td>12</td>
<td>83</td>
<td>1.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* \(\frac{1}{2}\) CO = 0.25% cholesterol supplement with 5% oil
† 2 C = 2% cholesterol supplement
† Index = average grade of thoracic lesions of all birds

The lesions discussed in this study are those occurring in the thoracic aorta of birds on cholesterol enriched diets. The grading varies from 0 to 4 on the basis of size, number and elevation of the atheromatous plaques. The grading was done without knowledge of the group to which the individual bird belonged in order to add objectivity to the data.

A. **Diets Containing 8 Per Cent Cholesterol.** Twenty-two chicks placed in individual cages were given a limited mash diet enriched with 8 per cent cholesterol for periods up to 15 weeks.

It was clear that these animals did not utilize the excess cholesterol for energy purposes even in the face of obvious caloric privation. Instead, cholesterol was deposited in the blood vessels and various organs, particularly in the liver. These results made it appear of value to repeat the experiment with lower cholesterol dosages so that the effect of diet limitation on cholesteremia and atheromatosis could be assayed in birds on less abnormal diets.

B. **Diets Containing 2 Per Cent Cholesterol.** Seventeen animals were given a normal mash
diet until the age of 5 weeks. At this time they were divided into two groups and placed on the cholesterol diets. One group of 5 animals received an unstinted mash diet containing 2 per cent cholesterol. A comparable group of 12 animals received the same diet in amounts approximately two-thirds of the unrestricted group. The weight gain of the chicks on the restricted diet averaged about two-thirds of that of the chicks on the free regimen. It can be seen from table 1 that the animals on the 2 per cent cholesterol limited diet had approximately the same level of plasma cholesterol (600 mg. per 100 cc.) and incidence and degree of atheromatosis as did their controls receiving the same mash in unlimited quantities. This was so despite the fact that the hungry birds received a notably reduced amount of cholesterol per bird. The reduced feed intake was evident in the retarded growth rate of these animals, in the diminished comb size, in the absence of gross body fat at autopsy and in the reduced testicular size.

C. Diets Containing 0.25 Per Cent Cholesterol. A similar pattern was seen in 6 chicks receiving 0.25 per cent cholesterol enriched diet in limited (two-thirds of control) amounts. At the end of 15 weeks of feeding, 3 of the 6 birds on the restricted diet had lesions with an average grade of 1.4, while none of the 5 birds on the full diet had lesions. The plasma cholesterol levels were similar in the two groups averaging about 140 mg per 100 cc. (table 1). Other findings such as weight (fig. 1), body fat, testicular development and comb size were similar to that seen in the groups on 2 per cent cholesterol mash.

Six control animals receiving an unlimited normal mash diet without the cholesterol supplement for periods up to 36 weeks were found to have plasma cholesterols averaging about 100 mg. per 100 cc. and no thoracic atheromatosis.

Discussion
The limited food intake clearly retarded the rate of growth of the chicks on this regime. Thus the chicks on the two-thirds diet gained weight at a rate approximately two-thirds that of the animals on the full diet. The retarded weight gain was evident in the smaller size of the birds. The influence of underfeeding on endocrine balance was evident throughout the experiment in the diminished comb and wattles development. There was little or no gross body fat. These hungry animals were more irritable than their well fed controls, pecking at once at anything that passed in front of their cages. When the daily allotment of food was placed in their trays, it was quickly eaten, leaving the food bins empty for most of the day. By contrast, the chicks with an abundant food supply ate continuously, although leisurely, throughout the day. Yet these well fed chicks consumed only one and one-half times as much food in the entire day as did the starved animals in an hour or so.

The cholesterol intake of the chicks in the 0.25 and 2 per cent cholesterol groups on the limited diet was approximately two-thirds that of their controls. However, the cholesterol taken per Kg. of chick was about the same for the two groups, inasmuch as the starved chicks were only two-thirds the weight of the well fed animals.

The plasma cholesterol levels were similar in the limited and in the well fed groups. The effect of exogenous cholesterol, all other conditions being equal, would therefore appear to depend on the ratio of cholesterol intake per Kg. of body weight. This relation is curvilinear, higher concentrations of cholesterol giving less effect per unit of this compound.

Chronic malnutrition is known to result in a general lowering in the activity of the pituitary gland and through this organ, a reduced activity of the endocrine system as a whole. This effect was apparent in the reduced comb and wattle size of the malnourished animals as compared with the well-fed controls. The size of the comb is known to reflect the activity of the testes in particular and to some extent the adrenal cortical activity. In conformity with this, the testicles were found at autopsy to be much smaller in the starved chicks. This apparent reduced activity of the pituitary and the endocrine system as a whole either had no notable effect on the hypercholesteremia or atheromatosis, or its effect was neutralized.

The animals on a restricted diet also demonstrated a decreased resistance to cold as shown by the fact that some of them succumbed to cold when the room temperature fell during
the night, while well fed chicks in neighboring cages did not. This effect may have been due to the lack of subcutaneous fat insulation against cold.

**Formulation**

The fact that the plasma cholesterol increases despite the inadequate caloric intake demonstrates that there is a limit to the extent to which ingested cholesterol can be degraded for energy purposes.

Our results clearly demonstrate that atherosclerosis can develop in chicks on a very strictly limited diet. Thus the belief that obesity is necessarily related to atherosclerosis is seriously challenged. Our data suggest that the degree of atherosclerosis may even be somewhat greater in the chicks on the restricted diet containing cholesterol. It becomes apparent that the atheromatosis is due to ingested cholesterol rather than to the amount of food taken. Firstbrook has recently challenged this interpretation on the basis of similar studies on rabbits. Utilizing multiple correlative techniques, he reported that cholesterol-fed rabbits receiving either ad libitum or limited diets showed a high net correlation between relative weight gain and the severity of experimental lesions. Statistical handling of the arbitrary judgment of the grades of atheromatous lesions is open to question since the significance of a change from grade 0 to 1 is much greater than, for example, from 3 to 4. Analysis of his published data from this point of view reveals that the incidence of atheromatosis is high in the rabbits which did not gain, or even lost weight on the experimental cholesterol diets. Firstbrook’s data may therefore be considered as supporting our thesis that cholesterol-induced atherogenesis proceeds unchecked even at reduced levels of caloric intake.

The relation between obesity and atherosclerosis apparently depends on the fact that caloric excess in Western nations often signifies an increased cholesterol intake. In other nations, un gover ned caloric intake with its consequent obesity may take place with little cholesterol in the diet and with a low incidence of arteriosclerosis. Therefore the presumed correlation between overweight and atherosclerosis is probably coincidental.

**Summary**

The effect of a limited intake of a cholesterol-enriched diet on the tendency to hypercholesteremia and atherosclerosis was studied in chicks. Limitation of dietary intake to two-thirds of that normally taken did not protect against the development of hypercholesteremia and atherosclerosis in these malnourished animals.

The tendency to atherosclerosis and hypercholesteremia is correlated with the cholesterol ingested per Kg. of body weight of the animal. Atherosclerosis is thus shown to occur in the starved animals and in the absence of hyperphagia or obesity.

Cholesterol apparently can be utilized to only a limited degree for energy purpose even in the face of severely restricted caloric intake.

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