Effect of Estrogen on Atherosclerotic Lesions in Various Arterial Segments of Cholesterol-fed Chicks

By

Takeo Kuroyanagi

(畔 柳 武 雄)

Prof. Okiyama’s Clinic, School of Medicine, University of Tokyo

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Until recently it was believed that atherosclerotic lesions of the aorta induced by hypercholesterolemia always faithfully reflected changes in other arteries. Thus intensive experimental analysis of factors reducing hypercholesterolemia and protecting the aorta against atherosclerosis were undertaken in the belief that such factors would also protect the coronary and other arteries against atherosclerotic lesions.

However, it is now known that after administration of estrogen coronary arteries differ in their behavior from that of aorta. While considerable attention has been directed to atherosclerotic lesions of coronary arteries in experimental animals, cerebral arteries have received little attention, in spite of the frequent incidence of cerebral vascular disease in man.

In the present study on chicks I have examined the effect of cholesterol feeding and hypercholesterolemia on the tendency to lipids infiltration and atherosclerotic changes in arteries of the brain, heart, kidney, lung, liver and spleen as well as of aorta. The effect of estrogen administration on these vessels was also studied.

METHODS

Newly hatched chicks of the DeKalb strain were given a normal mash diet for 12 weeks and then separated into three groups; Group 1 received mash diet to which 5% cotton seed oil was added, Group 2 received same diet supplemented with 2% crystalline cholesterol, and Group 3 received the diet of Group 2, plus the daily intramuscular injection of 1 mg. estradiol.

Measurements were made at intervals of feed intake, weight, comb size, and of the cholesterol and lipid phosphorous content of the plasma. The determination of plasma cholesterol was done according to Sperry-Schoenheimer’s method. Lipid phosphorous of the plasma was mea-
sured according to Man-Peters's method.

At autopsy the aorta and the coronary arteries were examined grossly and graded as unknowns by at least two experienced observers according to previously described method. Microscopic study of coronary arteries, arteries in the brain, kidney, lung, liver and spleen was carried out on each of these tissues stained by hematoxylin-Sudan IV. The microscopic grading system as follow:

0: No lesions
1: Occasional slight lipid infiltration in intima
2: Moderate diffuse lipid infiltration in intima and media
3: Severe diffuse lipid infiltration in intima and media with intimal cell proliferations and occasional plaque
4: Frequent plaques and intimal thickening with severe diffuse lipid infiltration in intima and media

A summary and average value was employed to demonstrate the grade of atherosclerotic lesions in each group.

**RESULTS**

**Group 1: Regular mash diet plus 5% cottenseed oil**

At 12 weeks of age, this group of 10 chicks was continued on the same mash diet they had received previously except that 5% cottenseed oil was added. These animals had weekly feed intakes of approximately 450 gm. per kg body weight. At sacrifice at 20 weeks (8 weeks on experimental diet) comb size averaged 108 sq. cm, determined as the product of the sagittal length multiplied by maximal height. The plasma cholesterol level averaged 160 mg. % and the lipid phosphorous 8.3 mg. %, making cholesterol / lipid phosphorous ratio of 19.

None of the 10 animals had atheromatous lesions in the aorta; six had very slight fibrotic lesions of the abdominal aorta without lipid infiltration.

Microscopic study of the thoracic aorta, intracerebral, coronary, intrarenal, intrapulmonary, intrahepatic and intrasplenic arteries showed no arterial lipid infiltration or atheromatous changes.

**Group 2: Cholesterol feeding**

This group of 10 animals was given the diet of preceding group except that 2% commercial crystalline cholesterol was mixed homogeneously in the oil containing mash.

Food intakes (342 gm. per kg. of body weight), comb indices (100 sq. cm.) and body weight at sacrifice were essentially similar to those of animals on the diet without cholesterol (Group 1).

At sacrifice the plasma cholesterol level of this group averaged 1415 mg. %; the lipid phosphorous averaged 14 mg. % and cholesterol / lipid
phosphorous ratio averaged 110.

Gross examination at autopsy revealed significant fat deposition in the abdominal viscera. The thoracic aorta showed moderate atherosclerotic lesions in all animals with an average grade of 2.1. Seven of 10 animals had minimal lipid infiltration of the abdominal aorta graded 1.2. Nine of the 10 animals showed involvement of coronary arteries on gross examination.

On microscopic examination all aorta showed lipid infiltration and atherosclerosis of moderate degree (average grade 2.7). The intima of coronary arteries showed severe lipid infiltration and frequent atheroma with average grade of 2.5. Nine of 10 animals showed moderate diffuse lipid infiltration in the intracerebral arteries with average grade of 1.8. No animals showed lipid infiltration in the intrapulmonary arteries. However, lipid deposition in the adventitia of the intrapulmonary arteries was seen in all animals. All animals showed slight lipid infiltration in intima of intrarenal arteries, being graded on the average as 1.0. Lipid infiltration of the intrahepatic arteries was usually slight, graded as 1.5. The intrasplenic arteries showed moderate diffuse lipid infiltration with average grade of 2.0.

Group 3: Cholesterol feeding plus estrogen administration

This group of 10 chicks had feed intakes approximately equal to that of the other two groups. At sacrifice, these animals averaged 2.4 kilograms. They demonstrated diminished comb indices, averaging 48 sq. cm., less than half that of previous two groups.

At sacrifice, the plasma cholesterol averaged 2430 mg. %, lipid phosphorous averaged 76 mg. %, and cholesterol/lipid phosphorous ratio averaged 32.

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<td>Feed-intake, Bodyweight, Comb Indices and Blood Chemistry</td>
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<td>Feed intake/kg. gm.</td>
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At autopsy these animals showed a greater degree of gross fat deposition in the viscera and enhanced yellowness of fat. Gross examination of the aorta showed atheromatosis rated as moderate, averaging grade 2.1. The coronary arteries were grossly free of lipid infiltration and atherosomatous lesions.

Microscopic examination of the aorta showed severe lipid infiltration in intima and media and atheromatous plaque formation with an average grade of 3.4. Microscopic examination revealed no lipid infiltration in the coronary arteries of 5 animals, while 5 animals showed slight lipid infiltration. Slight lipid infiltration of the intracerebral arteries was seen in 5 of 10 animals. The lipid infiltration in intima of intrarenal arteries was extremely uncommon, being graded as 0.5. The intrapulmonary arteries showed no lipid infiltration in all animals. The atherosclerotic lesions of the intrahepatic and intrasplenic arteries was graded as 0.8 and 1.9, respectively.

**DISCUSSION**

The results of Group 1 provided a base line showing that chicks fed a mash diet with a small oil supplement have normal cholesterol and lipid phosphorous level, and the cholesterol / lipid phosphorous ratio in normal range, with no lesions in aorta, coronary, intracerebral, intrapulmonary, intrahepatic and intrasplenic arteries. The fibrous lesions found in the abdominal aorta of some of the animals are generally recognized as normal variants depending on the age at sacrifice.

The present results demonstrate that the feeding of cholesterol produces atherosclerotic lesions not only in the aorta and coronary arteries but also in the intracerebral, intrarenal, intrahepatic and intrasplenic arteries as well. Katz and Stamler\(^5\) reported that the atherosclerotic
lesions in the pulmonary artery were produced by feeding of cholesterol. We also found atherosclerotic lesions in the stem of pulmonary artery; however, lesions in the intrapulmonary arteries were extremely uncommon and their atherosclerotic grade was very slight.

The low incidence of atherosclerotic lesions and very slight lipid infiltration in intrapulmonary arteries would be associated with low blood pressure. While the pressure in the main pulmonary artery is above 25/10 mm. Hg, the pressure in the smaller arteries is much lower, probably of the order of 12 to 15 mm. Hg. Thus it may be that damping of the systolic pressure may be sufficient to reduce the degree of lipid infiltration in the smaller arteries. This would support Anitschkow's\textsuperscript{6} concept that an increased blood pressure plays a role in disposing the arteries to become the seat of atherosclerosis.

Hypercholesterolemia induced by cholesterol feeding in chicks is fairly closely related with the degree of aortic lipid infiltration and atheroma-
tosis. When estrogens were given to produce the characteristic high blood cholesterol and lipid phosphorous values in chicks, the aortic atherosclerotic lesions became accentuated and also showed an increased deposition of carotenoid pigments. It is remarkable that the atherosclerotic lesions of aorta are more severe than those of other arteries. The aorta plays the important role as the surge tank of the arterial tree, expanding as it takes up the stroke output of each systole, and constricting during the diastolic run-off. It is possible that the recurrent stretching of the vessel may facilitate the rapid entry of lipids into the intima and media, under the driving force of the arterial pressure. Further the pressures in the aorta are usually higher than in other parts of the arterial tree, and this would enhance the tendency for the lipid infiltration into the vessel wall. Many of the arterial segments in organs have extraarterial support. This support may contribute to reduce the effective filtration pressure and thereby provide a relative protection against lipid infiltration.

The vessels examined in the course of present study may be divided into three groups on the basis of the effect of estrogen administration; 1) those in which atherosclerotic lesions were enhanced; 2) the arteries which were unaffected; 3) those in which the lipid infiltration was markedly reduced.

The atherosclerotic lesions of the aorta became accentuated by estrogen administration. This enhancement may be due to the higher hypercholesterolemia in estrogen group than in cholesterol-fed chicks.

The atherosclerotic changes in intrahepatic and intrasplenic arteries were unaffected by the estrogen administration.

The arteries of brain, heart and kidney were protected selectively against lipid infiltration by estrogen administration, in spite of the higher
hypercholesterolemia. This finding confirms Katz and Stamler's report that the coronary atherosclerosis is prevented by estrogen administration. These results may indicate that the intracerebral arteries and intrarenal arteries have similar behavior in the process of atherosclerosis with the coronary arteries and these three arteries differ in their behavior from that of aorta.

The mechanism of protection is not known. However, among the possibilities to be considered are

1) that these vessels are altered by the estrogen administration so that the lipid either cannot enter the vessel wall, or may be eliminated more rapidly from it,

2) that some changes are present in the cholesterol transport mechanism in blood,

or

3) that the blood pressure filtration effect is reduced.

It is known that the transport mechanism for lipids, cholesterol are affected by estrogen administration. Thus, in man the total cholesterol and beta-lipoprotein are reduced by estrogen administration. In present study on chicks the plasma lipid phosphorous increased to a much greater extent than the plasma cholesterol, with a lowering of the ratio of these two materials in the blood. The properties of the modified lipoprotein probably reduce their permeability in the arteries of the brain, heart and kidney. It is also possible that the ability of these vessels to eliminate lipid materials which have passed into the vessel walls is enhanced.

Evidence has accumulated to show that estrogen administration to cockerels produces a small but significant lowering of the blood pressure. This lowering of blood pressure by administration of estrogen may reduce the filtration pressure. However, a lowering of blood pressure should have an effect on all the arteries rather than only few selected arteries. Since the lesions in the aorta are more evident in estrogenized chicks, and since the lesions in the spleen and liver are unaffected, it would appear that this lowering of blood pressure by estrogen administration has no effect. Other factors must therefore be sought for the mechanism of estrogen protection of coronary, intracerebral and intrarenal atherosclerosis.

A common feature of the arteries of the heart, brain and kidney is a significant extraarterial support pressure. Thus, the intracerebral arteries are supported by the cerebrospinal fluid pressure of perhaps 10 mm. Hg. In the smaller arteries of the brain this transmural reduction in pressure may also reduce the rate of filtration of materials through these vessels. If the blood pressure were also reduced, it might account for the protective action of estrogen on these important channels.
The main coronary arteries of chicks\textsuperscript{14,15} course through the ventricular muscle. In this site they receive the support of intraventricular pressure and pressure from muscle contraction. This support pressure may reduce the filtration pressure markedly and protect coronary arteries against atherosclerotic lesions.

**CONCLUSION**

1. The aorta reflects the atherosclerotic conditions in most of the arterial system of the body, when cholesterol feeding induces hypercholesterolemia and atherosclerosis.
2. The intrapulmonary arteries are exempted, possibly because of the low pulmonary arterial pressure.
3. Estrogen separates the arteries of the body into three groups, when it is administered to cholesterol-fed chicks.
   i. The aorta becomes more severely involved,
   ii. The intrahepatic and intrasplenic arteries remain relatively unaffected by estrogen administration,
   iii. The intracerebral, coronary and intrarenal arteries are protected against atherosclerotic lesions.

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**References**