

Histochemical Studies of Fetal Arteries of Koreans with Special Reference to Atherogenesis in Adults

Pil Soo Shin and Dong Sik Kim

*Department of Pathology
Yonsei University College of Medicine, Seoul, Korea*

(Received for publication: November 29, 1963)

ABSTRACT

In view of the sparsity of report on normal and abnormal pattern of the major arteries during fetal life, the authors undertook investigation of the aortas, coronary arteries, renal arteries, and the umbilical arteries of 76 Korean fetuses, ranging from 2¹/₂ months to full-term. A normal pattern and its evolution of the intima, media, and the adventitia was described. The P. A. S. positive substance was most abundantly found in the media of the umbilical arteries, medium amount in the media and intima of the renal arteries and the aortas, and lesser amount in the media of the coronary arteries. A surprisingly high incidence of the alteration of the internal elastic membrane of the aorta simulating to the early lesions of atherosclerosis in adult and neonatal life was observed. In eleven instances, microcystic degeneration of the inner media of the aorta was observed, and its relationship to the idiopathic cystic medial necrosis and dissecting aneurysm was discussed.

INTRODUCTION

Although a voluminous literature has accumulated concerning atherosclerosis at present there is no unified conception of its etiology and pathogenesis. The theories and hypothesis as proposed up to now include the following concepts:

1. The natural occurrence of atherosclerosis is observed only in human beings and spontaneous atherosclerosis seldom develops in cats, dogs, rabbits, monkeys and in wild animals.

2. Atherosclerosis is rarely, if ever, encountered at birth.

3. The occurrence of atherosclerosis parallels rising age, and is almost constantly found among the older age group.

4. During young adult life, it is preponderantly a male disease but, with increasing age, the sex difference disappears, particularly so after the menopause.

5. In the atherosclerotic lesions a large amount of lipids particularly cholesterol and esters are found. The composition and distribution of lipids in atheroma closely parallel that found in the serum, indicating a deposition of serum lipids creates the atheroma.

6. Atherosclerosis tends to develop at a point of stress, and hypertension is likely to be an important factor.

However, no one theory satisfactorily explains the whole picture of atherogenesis, but many factors must be operating together. It is known also that atherosclerosis is not only a phenomenon of the aged, but also that its early or antecedent form may be found at a younger age.

Stryker(1946) and Cochrane and Browden(1954) reported observations of calcific changes in coronary and other arteries in still-born and newborn babies. The deposition of calcium was noted mainly at the internal elastic membrane. Grunwald(1949) examined 21 cases of still-born and new-born infants who died 3 days after the birth, and observed localized medial

degeneration in the coronary arteries, and proliferative obliterating changes in the pulmonary arteries. Wolman(1950) also reported similar findings. Weinberg(1943), Kelly and Anderson(1956), Gagnon and dela Querriere (1958) reported a finding in infants, fibrous thickening of the endocardium, the so-called "endocardial fibroelastosis". Mckusick (1958) investigated congenital factors in dissecting aneurysms and idiopathic cystic medial necrosis. He stated that cystic medial necrosis was often associated with congenital heart disease, and a hereditary and familial tendency to have chemical disturbances of the ground substance in idiopathic cystic medial degeneration.

These observations indicate a significant relationship between the normal and altered pattern of arteries in a fetus and infant, and pathologic lesions found in adults. Therefore a detailed investigation of arteries at an early period of life is indicated. Moon(1957) made histochemical studies of coronary arteries in 24 fetuses and observed rupture of internal elastic membranes in a 4 to 6 weeks premature still-born fetus, but no evidences of fraying, reduplication of internal elastic membrane, or of endothelial proliferation were noted. However, similar studies in infants and during early childhood showed all types of changes which corresponded to the early lesions of atherosclerosis in adults.

On account of all these above facts, the authors thought that it would be worthwhile to investigate normal and abnormal patterns of fetal arteries of Koreans. Such investigation has been carried out yet.

MATERIAL AND METHODS

Materials consisted of 76 fetuses ranging from 2½ months to full term. Distribution of fetuses according to fetal age was as follow.

Mo.	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	9	9½	10
No.	1	1	1	12	3	14	2	16	3	12	2	3	2	1	3

The aorta, coronary arteries, renal arteries, and umbilical arteries of each fetus were examined microscopically. Sections of aorta were taken from ascending, thoracic, and abdominal portions, sections of coronary arteries from anterior descending and

circumflex branches close to the ostium, sections of the renal arteries from the hilum of the kidney, and sections of the umbilical arteries at any level. Sections were embedded in paraffin after fixation in 10% formalin. Hematoxylin-Eosin, Periodic-Acid-Schiff stained, and Verhoeff's elastic stained slides were made. Sudan IV stains were made on all tissues after frozen section. Microscopic examinations were made.

RESULTS

Observations were divided in two major groups of findings normal and abnormal. Both normal and abnormal findings were recorded in aortas, coronary arteries, renal arteries, and umbilical arteries according to different staining and listed according to the age of the fetus.

Normal findings

The intima of aortas, coronary arteries, and renal arteries consisted of single layer of endothelial cells and internal elastic membrane throughout fetal life. Umbilical arteries, however, lacked a distinct internal elastic membrane. In all four arteries, the endothelial cells in early fetal life were tall cuboidal with evidence of secretory activity being found in the coronary artery, cells became low cuboidal and even flattened as the fetal age increased. A well demonstrated internal elastic membrane was observed in the aorta at the 3rd fetal month and in the coronary and renal arteries the 3½th month. The internal elastic membrane was well demarcated, uniform, and thin in early fetal life, and then gradually became thicker as age increased. However, it was several times thicker than the elastic fibers in the media. It was a continuous membrane, but had many small fenestrations, which sometimes gave the impression of a chain of interrupted fragments. Also it was markedly corrugated; a finding most prominent in the renal arteries. No demonstrable connective tissue was noted in the intima throughout fetal life.

The media showed an abundance of elastic fibers in the aorta even in a 2½ month old fetus, but their outline was rather indistinct at early age although it became clearer later. The elastic fibers in coronary and renal arteries were scanty and very

fine in thickness throughout the fetal life, and were negligible in the umbilical artery. The media of the umbilical artery consisted mainly of a muscular layer.

The adventitia of aortas, coronary and renal arteries was indistinct during early fetal life, and then slowly formed a loose, ill-outlined thick, edematous connective tissue from the surrounding mesenchymal tissue. As the age of the fetuses increased this gradually condensed to become a thinner and better defined tunica with collagenization and incorporation of the fine elastic fibers derived from the outer media. During the early fetal life in the aorta, there was definite transformation of the inner adventitia into the outer media.

The vasa vasorum of aorta was observed during the fifth fetal month as they branch into or more become incorporated with the adventitia the age increased. But even in the full-term fetus, they did not reach beyond the 1/3 of the media.

At several occasions, the branching point of an artery from aorta was observed. At the outlet, the internal elastic membrane became a little thinner and then was directly continuous with the internal elastic membrane of branched artery together with its endothelial layer. However, the elastic fibers of the media emerged and polymerized to become fewer and to join the media of the branched artery. The adventitia continued directly along the media without any notable change:

P. A. S. Staining

Throughout entire fetal life, P. A. S. staining showed exceedingly abundant P. A. S. positive materials in the media of the umbilical artery. distributed mainly in the muscle cells and fibers, and a small amount in granular or fibrillary form in interstitial area. In the aorta, coronary, renal arteries, P. A. S. positive materials were observed in the internal elastic membrane, and the media, from early fetal life, and in the adventitia in later fetal life. The P. A. S. positive materials in the media were mostly located in elastic fibers, but also at an early life were found in muscle cells and the interstitial area. However, in the later fetal life, the materials noted mostly in elastic fibers.

Sudan IV stains showed no demonstrable sudano-

philic material in all four arteries throughout the entire fetal life.

Abnormal Findings

Abnormal findings were observed mostly in the aorta. They were limited to the intima and inner media and consisted of proliferative intimal thickening, fraying, splitting, reduplication, disruption of the internal elastic membrane, and microcystic degeneration of the inner media.

A proliferative intimal thickening was observed in 10 fetuses, 3 at the 5th month, 6 at the 7th month, and 1 at the 9th month. The involvement was usually a focal protrusion into the lumen which consisted of fibrous connective tissue.

Fraying of the internal elastic membrane was observed in 6 fetuses, 2 at the 5th month, 1 at the 6th month, and 3 in the 7th month. Four of the membranous changes were associated with intimal thickening.

Splitting of the internal elastic membrane was observed in 3 fetuses, 1 at 6 $\frac{1}{2}$ th month, 2 at 7 $\frac{1}{2}$ th month. This splitting was not associated with any intimal thickening or with fraying of the internal elastic membrane.

A reduplication of the internal elastic membrane was found in only one fetus, at the 7 $\frac{1}{2}$ th month and was associated with a splitting of the membrane.

Disruption of the internal elastic membrane was observed in 4 fetuses, 2 at the 7th month, one at the 7 $\frac{1}{2}$ th month and one at the 8th month.

Microcystic degeneration of the media was observed in 11 fetuses, 2 at the 5th month, 3 in the 7th, 5 in the 7th, and one in the 9th fetal month. The cysts were found in the subintimal part of the inner media as irregular spindle shaped, isolated, or communicating cysts. The contents were faintly basophilic and amorphous by a hematoxylin and eosin staining, and were negative to P. A. S., elastic, and Sudan staining.

Elastic fibers of the media at the lesion site became thinner, frayed, disrupted, and separated widely.

In only one fetus, at the 7 $\frac{1}{2}$ th month, a focal calcification in the media of coronary artery was observed. Otherwise, no abnormal findings were noted in the coronary arteries.

Three fetuses showed an acute non-specific inflammatory reaction in the umbilical artery with early thrombosis of the lumen, 1 in the 6th month, and 1 in the 7^{1/2}th month. One of fetus at the 5th fetal month showed a single umbilical artery as well as inflammation.

No abnormal findings were observed in the renal arteries.

DISCUSSION

A surprisingly high frequency of abnormal findings was observed in the aorta during fetal life. These consisted of proliferative intimal thickening, disturbances of internal elastic membrane such as fraying, reduplication and disruption, and microcystic degeneration of inner media. These are exceedingly interesting findings in view of the fact that they are quite similar to those found in the various stages of arteriosclerosis observed in neonatal and adult life. In the present investigation these lesions were found only in the aorta. The only lesion found in the coronary arteries was a focal calcification of the media in one fetus. Moon (1957) in his histochemical investigation of coronary arteries found rupture of internal elastic membrane in two of 24 fetuses, and a higher frequency of early arteriosclerotic lesions in the coronary arteries of 52 infants from 0 to two years of age. Gruenwald, (1949), Wolman (1950), and Cochrane and Browden(1954) observed degenerative changes in the coronary arteries of still-born fetuses. Moon and Reinhart(1952) also observed simultaneous deposition of mucopolysaccharide at the area of proliferative intimal thickening using a colloidal iron staining method. However, the P. A. S. stained tissue in present investigation did not always show intimal change. This discrepancy is probably due to a difference of staining between P. A. S. and colloidal iron stains.

Apparently P. A. S. staining failed to demonstrate acid mucopolysaccharide as evidenced by a negative stain of Wharton's jelly in the umbilical cord. The most prominent alterations of internal elastic membrane in the lesion, which the authors thought to be due to early arteriosclerotic changes in the present study, agrees with the previous reports by Wolkoff(1923), Ehrlich, de la Chappelle, and

Cohn(1931), Fox(1933), Gross, Epstein, and Kugel (1934), Blumenthal, Lansing, and Gray(1950), Moon and Reinhart(1952), Lindsay, Chaikoff, and Gilmore (1952), Lansing, Rosenthal and Dempsey(1952), Moon (1957), Kim(1961). They have emphasized that the most important change in the pathogenesis of arteriosclerosis is a degeneration of the internal elastic membrane.

Microcystic degeneration of the inner media in the present investigation is reminiscent of those changes of idiopathic cystic degeneration of media found in the adult aorta, and perhaps this is an antecedent lesion of cystic medial necrosis. Mckusick (1958) reported that the medial cystic degeneration is a cause of dissecting aneurysm and he tried to investigate its genetic nature. In this connection, further investigation of micro-cystic change in the inner media of the aorta during fetal life may throw light on the pathogenesis of dissecting aneurysms of the aorta.

Three instances of acute inflammatory changes in the umbilical artery may due infected amniotic fluid.

In view of the sparsity of reports on the normal histochemical structure of the major arteries during the fetal life, according to the age of fetus, in spite of many reports on the genetic aspect of these arteries in embryology, the authors believe that this report on the normal developmental course of structure of major arteries during fetal life may serve as a guide in future investigations.

CONCLUSIONS

1. Histochemical and morphological investigations of aorta, coronary, renal and umbilical arteries of 76 fetuses, ranging from 2^{1/2}month to full-term, were made and tabulated according to the age of the fetuses.
2. P. A. S. positive substance was most abundantly found in the media of the umbilical artery, a moderate degree in the intima and media of the renal artery and aorta and to somewhat lesser degree in the coronary arteries. These substances were thought to be a neutral mucopolysaccharide.
3. A rather high incidence of lesions resembling those of arteriosclerosis in adult were observed in

the aorta. These were a proliferative intimal thickening fraying, splitting, reduplication, and rupture of the internal elastic membrane. These were thought to be the antecedent or primordial lesion of arteriosclerosis in neonatal and adult life. The most prominent changes were alteration of internal elastic membrane. Its significance in the genesis of arteriosclerosis is discussed.

4. In eleven instances, a microcystic degeneration of inner media was observed, and the possible relationship between cystic medial necrosis of the adult and a causal relationship to the occurrence of a dissecting aneurysm is discussed.

5. None of the 76 fetuses showed a demonstrable lipids deposition in the arterial wall. However a focal medial calcification was observed in the coronary artery in the one fetus. Incidental findings included three cases of umbilical arteritis which were thought to be due to infection of amniotic fluid.

REFERENCES

- Blumenthal, H. T., Lansig, A. I. and Gray, S. H.: *Am. J. Path.* 26:989, 1950.
- Cochrane, W. A. and Browden, D. H.: *Pediatrics*, 14:222, 1954.
- Ehrlich, W., de la Chappelle, C. E. and Cohn, A. E.: *Anat.* 49:241, 1931.
- Fox, H.: In Cowdry E. V.: *Arteriosclerosis*, Chap. 6:153, Mac Millan Co., New York, 1933.
- Gagnon, J. and Dela Querriere, L.: *Rev. Canad. Biol.* 17:512, 1958.
- Gross, L., Epstein, E. Z. and Kugel, M. A.: *Am. J. Path.* 10:253, 1934.
- Gruenwald, P.: *Am. Heart. J.* 38:539, 1956.
- Kelley, J., and Anderson, D. H.: *Pediatrics*:18:539, 1956
- Kim, D. S.: *Yonsei Med. J.*, 2:42, 1961.
- Lansing, A. I., Rosenthal, T. B., Alex, M. and Dempsey, E. W.: *Anat. Rec.* 114:555, 1952.
- Lindsay, S., Chaikoff, I. L. and Gilmore, J. W.: *Arch. Path.* 53:281, 1952.
- McKusick, V. A.: *Ann. Int. Med.* 49:556, 1958.
- Moon, H. D. and Rinehart, J. F.: *Circulation* 4:481, 1952.
- Moon, H. D.: *Circulation* 16:263, 1957.
- Stryker, W. A.: *Am. J. Path.* 22:1007, 1946.
- Weinberg, H. A.: *Am. J. Dis. Child* 68:405, 1944.
- Wolkoff, K.: *Arch. Path. Anat.* 241:42, 1923.
- Wolman, K.: *Am. J. Med. Sc.* 220:133, 1950.



Fig. 1. Umbilical artery of a four months old fetus, showing abundant P. A. S. positive substance at the media. P. A. S. stain, $\times 300$.

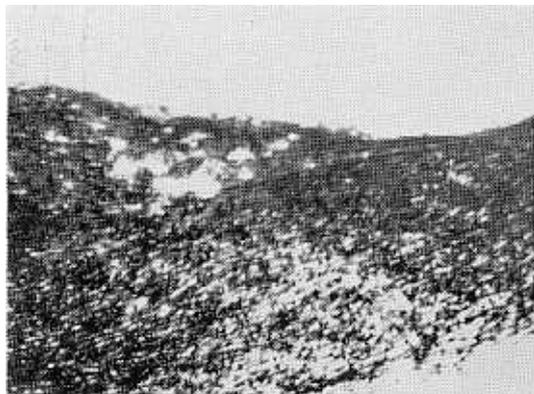


Fig. 2. Aorta of a five months old fetus, showing focal hyperplastic intimal thickening over the internal elastic membrane. Verhoeff's stain, $\times 300$.

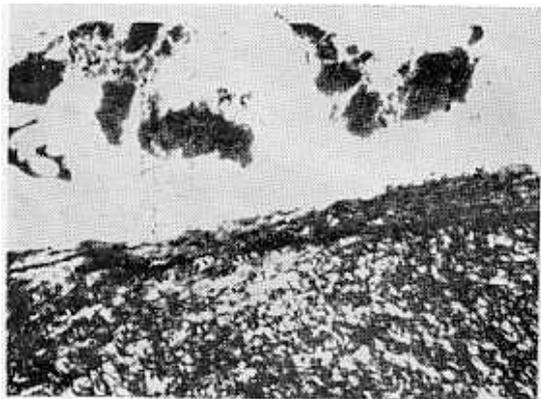


Fig. 3. Aorta of a seven months old fetus, showing fraying and reduplication of internal elastic membrane. Verhoeff's stain, $\times 200$

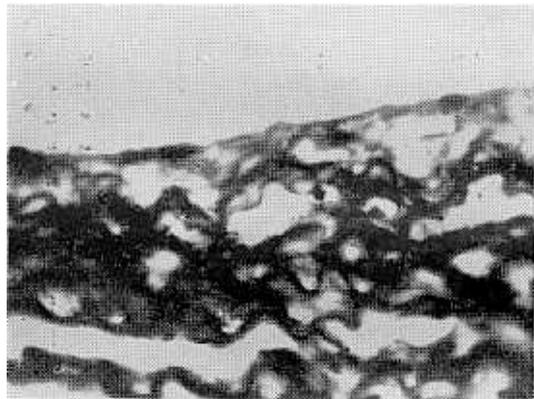


Fig. 4. Aorta of a seven months old fetus, showing focal intimal thickening and subintimal cystic degeneration of inner media. P.A.S. stain, $\times 300$



Fig. 5. Aorta of a 9 months old fetus, showing focal intimal thickening, swelling and reduplication of internal elastic membrane. Verhoeff's stain, $\times 200$.

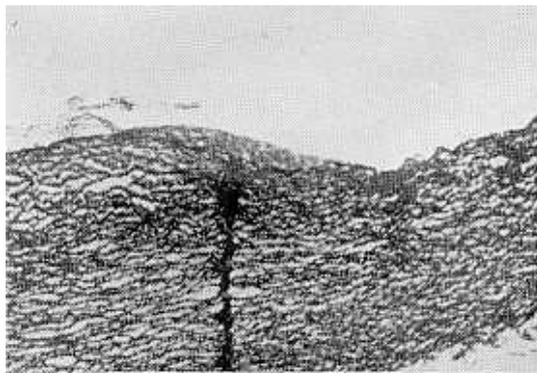


Fig. 6. Aorta of a six months old fetus, showing marked subintimal cystic degeneration of inner media. H & E stain, $\times 300$.