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Contributions to the study of the foetal development of physiological intimal thickening in the human uterine artery

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The morphological study of the development of "intimal thickenings" of the human uterine artery in physiological condition was performed on 72 uterine arteries obtained from foetuses from the 12th week of gestation up to birth.

Our results indicate that intimal thickening is formed by the migration and displacement of mesenchymal cells around the site of origin of collateral vessel from uterine mesothelium. These cells firstly differentiate into the myoblasts and then into the myocites.

During the development the internal limitans membrane separates the intimal thickening from the tunica media and the elastic fibres appearing inside possessing a muscle-elastic nature.

The function of intimal thickenings is the regulation of local blood flow by means of the control of myocitic contractile capacity; these cells play a fundamental role in endothelium-intimal smooth muscle cell contact.

key words: uterine artery, intimal thickening, human foetus

INTRODUCTION

One of the most characteristic features of the structure of the uterine artery is the presence of "cushions" or "arterial pads", which enables the fitting of the arterial wall to changes in the blood flow.

The numerous studies performed on the uterine artery have revealed characteristics of the structure (muscle-elastic), morphology (simple, columnar, polypoid or valvular), distribution (eccentric or diffuse) and localisation (at the place of emergence of the collateral branches or in the vascular circumference) of intimal thickenings [3–5, 9, 10, 21]. The term "intimal thickenings" — the result of unified different nomenclature made by the American Heart Association [1] in a consensus document — concerns the structure taking part in the regulation of blood flow in physiological conditions, but in pathology it is the main place of atherosclerotic changes.

MATERIAL AND METHODS

The studies were performed on 72 human foetal uteri obtained from legal autopsies in order to describe the structural development of "intimal thickenings" at the collateral branches from the 12th week of gestation up to birth.

The age of the embryos was estimated according to the criteria proposed by O'Rahilly and Muller, based on different measurements (maximum length, skull-heel length, biparietal diameter, abdominal circumference, cephalic circumference, and corporal weight). These measurements were compared with the case histories and echography, if they were avail-

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able. Afterwards we dissected the genital apparatus and its mesothelium.

Specimens were sectioned into 2-mm-thick pieces and fixed in 10% formaldehyde solution. After dehydration through graded alcohols, they were embedded in paraplast and cut on the Leitz microtome. Sections were stained with Martins' trichrome and Orcein and Verhoeff methods to identify the elastic tissue.

The pieces for semithin and ultrathin sections were fixed in 2.5% glutaraldehyde in Milloning buffer and embedded in araldite. 1-μm-thick serial sections were stained with the Toluidin blue method.

The ultrathin sections were postfixed with 1% osmium tetraoxide, dehydrated through graded alcohols and cleared in propylene oxide, and then contrasted with 2% aqueous solution of uranyl acetate.

RESULTS

During the 12th to 14th weeks of gestation, great angioblastic activity occurs in the uterine mesothelium, with the formation of collateral vessels by means of the confluence mechanism and *in situ* fusion of blood islets (Fig. 1).

When the artery and its collateral branches were strengthened, we could see angiotrophic phenomena with migration of mesenchymal cells. In the 16th week of gestation the mesenchymal cells congregated near the place of emergence of the collateral vessel, this observation being the first sketch of the future intimal thickening (Fig. 2).

Later, these cells differentiated into the myoblasts (in the 17th week), characterised by the presence of a spherical nucleus, prominent nucleolus, loose chromatin and a elongated cytoplasm, and — at the end — into the myocites (in the 18th week) with spindled cytoplasm, elongated nucleus and a clear nucleolus.

The development of the internal limitans membrane in the arcuate branches made the intimal muscle cells independent of those in the tunica media. At this stage the intimal thickening was isolated by the internal elastic lamina, lying close to the vascular lumen and covered by endothelial cells.

When the intimal thickening became greater, elastic material appeared inside. The elastic fibres were arranged as thin undulated laminas found in the basal area of the thickening.

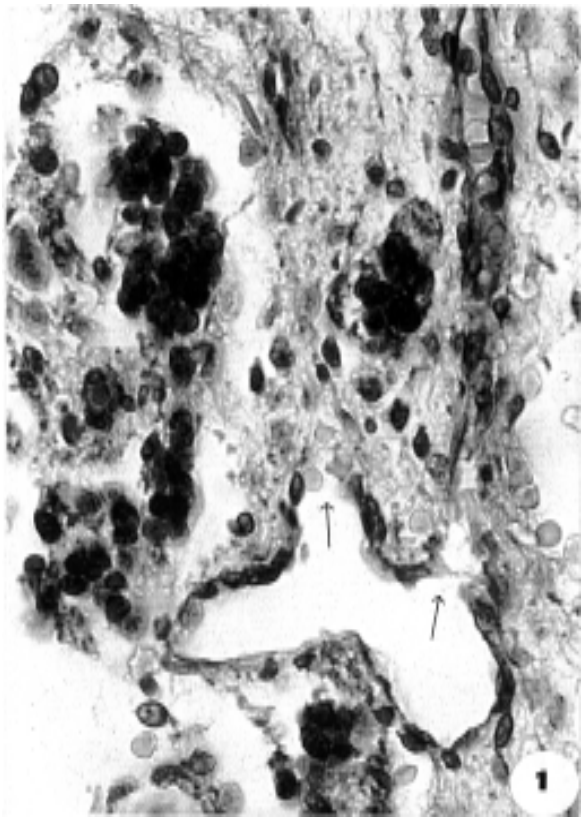

Figure 1. 12 weeks of gestation — the uterine mesothelium thicknesses are developing collateral vessels (arrows), by means of confluence and fusion of blood islets. Orcein × 750.

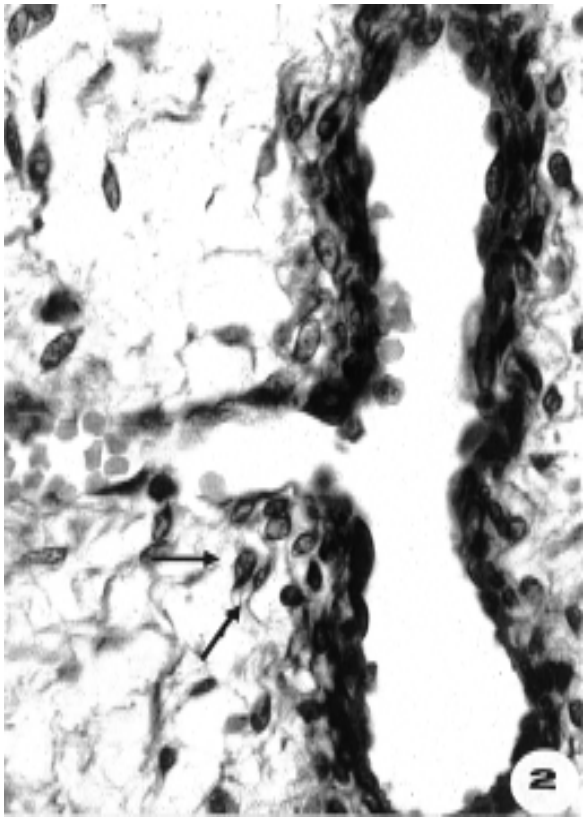

Figure 2. At 16 weeks the intimal thickening highlights the migration of mesenchimal cells (arrows) around the emergence of the collateral vessel. Orcein × 750.

At the end of the gestation, the intimal thickening showed two different areas: the inner one – lying near the lumen, rich in smooth muscle cells, and the outer one — with elastic fibres and longitudinally arranged muscle cells (Fig. 3).

We have found different morphological variations of the intimal thickenings: among them — the simple was the most frequent and was represented by small bulkiness in the part of the vascular circumference. Additionally, we have also observed two other types: 1) a valvular, characterised by cupola-shape folds projecting into the vascular lumen and with smooth muscle cells inside, and 2) a polypoid, formed by the prolapse of the media pushing the internal elastic membrane and the intima into the lumen.

Electron microscopy has confirmed our observations. In the 21st week of gestation the intimal thickening at the level of the exit of the collateral branch possessed the internal limitans membrane separating the tunica intima from tunica media (Fig. 5). In its inner part we could observe smooth muscle cells of synthetic phenotype, and the existence of a contact for simple myoendothelial apposition.

In the 32nd week of foetal life there was an arcuate collateral branch arising from the main vessel (Fig. 4) and the internal limitans membrane passing from the main vessel to the collaterals without interruption.

We have seen atherosclerotic pathological thickenings neither of the main vessel nor of collateral arcuate branches in any studied case.

DISCUSSION

Our findings show that between the 12th and 14th weeks of foetal life great angioblastic activity occurs in the uterine mesothelium, with characteristic formation of collateral branches originating from the main vessel. When the structure of the uterine artery is consolidated around the emerging collateral branches, there is a great number of mesenchymal cells lying near that place — being the first sketch of the future intimal thickening.

These cells migrate to a place around the bifurcation of the main vessel. The mechanism of this migration is still unknown, however, the platelets derived growing factor (PDGF) would take the crucial role in this process. PDGF stimulates the migra-

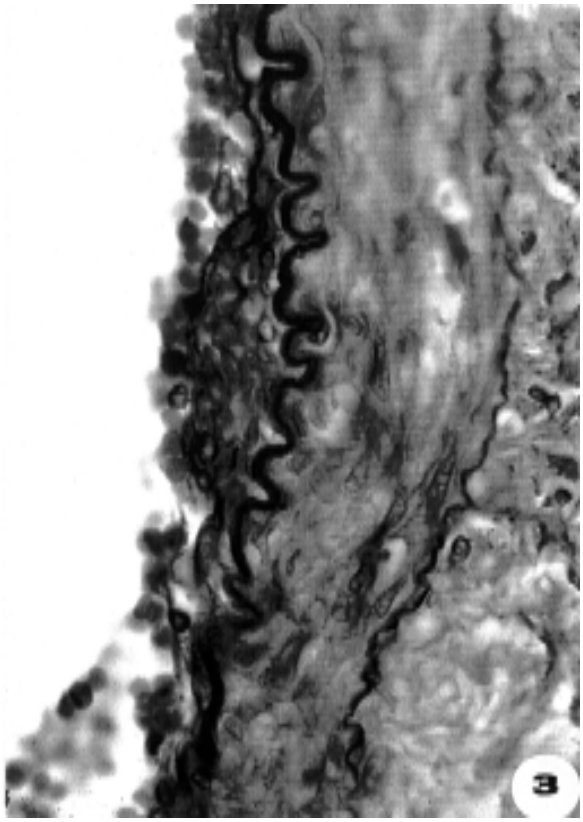

Figure 3. Intimal thickening in a newborn girl, showing preferential longitudinal distribution of smooth muscle cells. Orcein × 750.

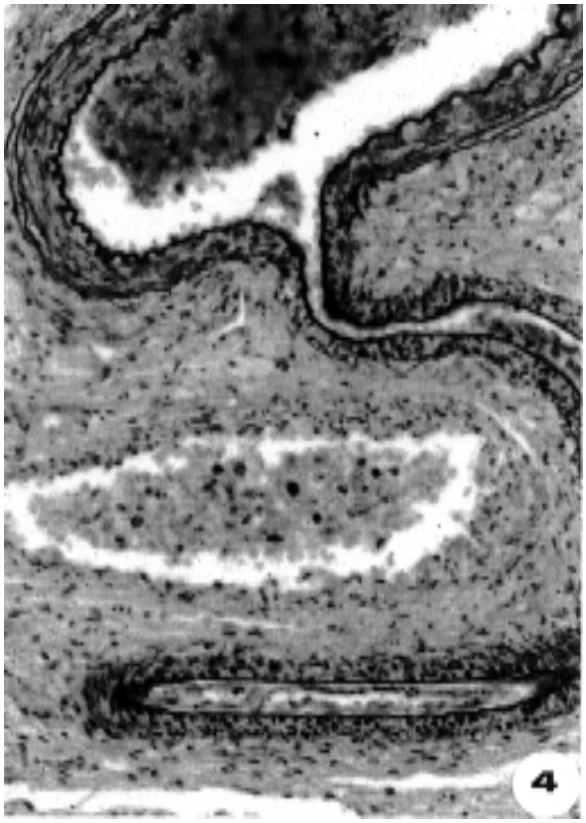

Figure 4. Panoramic view of main vessel and the emergence of a collateral branch, in a foetus of 32 weeks. Note how the internal limitans membrane continues without interruption from main vessel to collateral branch. Orcein × 25.

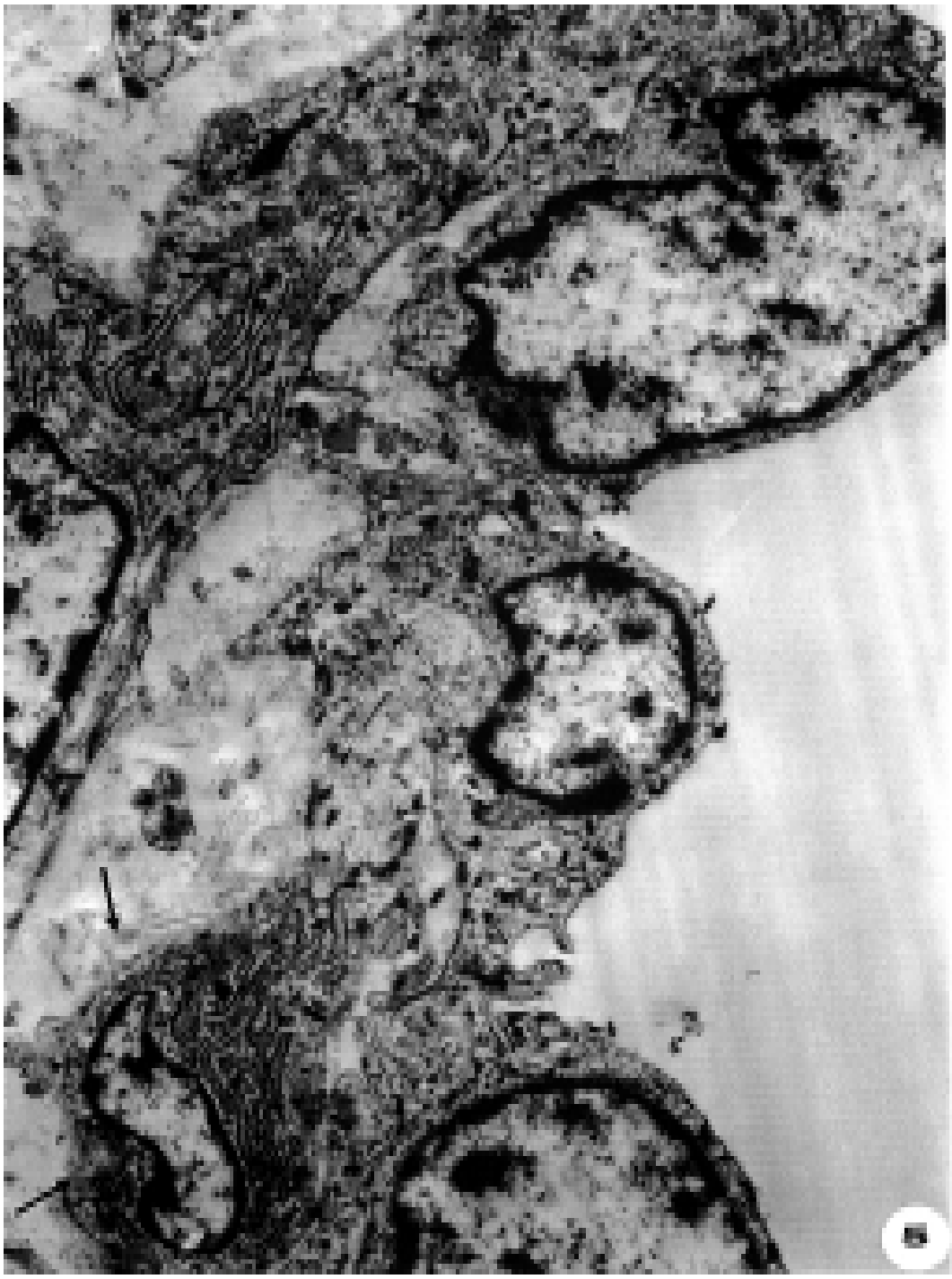

Figure 5. A physiological intimal thickening in the collateral branch origin. Note the presence of the internal limitans membrane separating tunica intima from tunica media and of a smooth muscle all of synthetic phenotype (arrows). Uterine artery of 21 weeks of development. X 5000.

tion of mesenchymal cells to the endothelium and once they contact, the endothelial cells release a transformation growing factor (TGF-P), that promotes the differentiation of mesenchymal cells to smooth muscle cells.

The intrauterine formation and evolution of the intimal thickenings, called "cushions", have been studied in different arteries by Robertson [12, 13]. This author has worked on the coronary, brachial, femoral, radial, popliteal and posterior tibial arteries, and

found, that in foetal life, the intimal thickenings were present only at the place of emergence of the collateral branches. Later, Velican and Velican [20] described the chronology, topography and structure of "cushions" in the coronary arteries. According to these authors, the first changes in the structure of the arteries were seen in the fourth month of foetal life and consisted of the migration of smooth muscle cells into the subintimal space, accompanied by reduplication, fragmentation and interruption of the internal elastic membrane. The intimal thickenings always developed in the same localisation (the place of the collateral branches' emergence) and constituted from a third to a half of the vessel's circumference.

According to Rapola and Pesone [11], the intimal thickenings are the substratum of the atherosclerosis development, while Matonoha and Zeichmeister [8] described them as an adaptation of the arterial wall to haemodynamic stress.

Vessels with short vital life, such as the umbilical artery and the ductus arteriosus, develop diffuse intimal thickenings that, due to their histochemical and structural characteristics, resemble the intimal thickenings of adult arteries. In these arteries during the development, the initial stages of the atherosclerotic plaque can be observed [15, 23].

As shown in figures, we have observed the presence of physiological devices of blood flow regulation in the foetal uterine artery, at the place of emergence of the collateral branches. They have a muscle-elastic structure and can adopt different morphology, from small bulkiness in part of the vascular circumference to columnar, polypoid or valvular "pads".

According to us, the elastic fibres of the intimal thickening are secreted by the smooth muscle cells of synthetic phenotype in the same way as the elastic material in the media of prenatal arteries [22]. According to Sasaguri et al. [14] and Li et al. [6] the synthesis of elastin in foetal vessels would be involved in the evolution of the intimal thickening with a change to a contractile phenotype of smooth muscle cells. The ultrastructural study shows the presence of a smooth muscle cell with synthetic phenotype in the intimal thickening, which confirms the elastic material formation.

The intimal thickenings are local regulators of the uterine blood flow, by allowing greater or smaller perfusion, depending on the contractile capacity of the smooth muscle and in answer to different substances, such as the catecholamines, oestrogens, vasoactive drugs and nitric oxide, that would pass bidirectionally between the lumen and the thickening. The observations of Sosa-Melgarejo and Berry [16], concerning the aorta of the human foetus, as well as Beny and Pacicca [2] and Marchenko and Sage [7] — in experimental animals, have shown that in the endothelium contacts between endothelial cells and smooth muscle fibres play an important role in the regulation of the muscle cells activity.

We also think that intimal thickenings are one more factor predisposing to the atherosclerotic degeneration, due to the deformation of the internal limitans membrane that they cause and the thin endothelium that covers them.

The presence of atherosclerotic plaques, so usual after the fourth decade of life, is very rare in foetal uterine arteries and is always in relation to additional pathological processes. Our observations have not revealed the presence of atherosclerotic thickenings either in the main vessel or in the arcuate and radial collateral branches. This fact is confirmed by Stepanov and Sapozhnikov [18], who did not observe intimal thickenings in the prenatal uterus.

In our material the formation of physiological intimal thickenings occurs *in situ* due to the differentiation from mesenchymal cells to smooth muscle cells; afterwards, the development of the inner elastic membrane isolates it from the media. In areas when the elastic laminas do not develop, as in transition segments and curvatures, the intimal thickening constitutes a unity with the media. These observations are in accordance with Stary et al. [17] concerning postnatal arteries. This mechanism is different from the formation of pathological thickenings, described by Thyberg [19] in the following events: changes in the phenotype of the smooth muscle cells from contractile to synthetic in the media, followed by migration of these cells to the intima by the fragmentations and breaking of the inner limitans membrane. Then the smooth muscle cells secrete an extracellular matrix that increases the thickness of the layer wall where muscle cells redifferentiated to a contractile phenotype.

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