DEVELOPMENT OF THE CONNECTIVE TISSUE STROMA IN THE FETAL PORTION OF THE HUMAN PLACENTA

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Of all the placental tissues, the connective tissue framework of the chorion is the one least investigated. The attention of research workers has been drawn principally to the epithelium and, to a lesser degree, to the vessels of the fetal portion. Only within the last few years have there appeared studies presenting the characteristics of the connective tissues as seen in early human embryos [1, 3]. These investigators state that the earliest extraembryoblastic human mesenchyme (in the initial postimplantation presonite period at about the 13-15 day stage) is divisible into the amnionic and chorionic. Soon reticular fibers appear between the elements of the chorionic mesenchymal syncytium transforming this structure into reticular tissue. Within this latter, blood islets form – the commencement of intravascular hematopoiesis.

Information in regard to the connective tissue components, especially the cellular elements, of the human chorion in the later stages of development is quite scanty. Ulesko-Stroganova indicates alterations in the mesenchymal chorionic stroma as gestation progresses [4], the early undifferentiated connective tissue, quite embryonal in appearance, being (after the fourth month) changed by the development of a large amount of collagenous fibers. Indications of a considerable collagenization in the stroma of the chorionic villi in the later developmental stages are present in other papers also [2, 5]; however, none of these authors suggest that there occur changes in cellular elements of the villous stroma.

In so far as metabolic exchanges between the mother and the embryo require the participation of the chorionic connective tissue, it seemed desirable to undertake a systematic study of the fetal connective tissues composing the placenta and examine them at various stages of their development.

EXPERIMENTAL PROCEDURES AND THE RESULTS OBTAINED

As our material for study, we took mature human placentas and chorions at various stages of pregnancy (from 5 to 16 weeks). This material was used only after we had satisfied ourselves that there had been no pathological changes in the fetal membranes. The tissues were fixed in Zenker-formol and 12% neutral formalin; Heidenhain hematotoxylin or azure-eosin was employed to stain the cellular elements; the Mallory method was used to demonstrate the interstitial fiber substance.

At a stage corresponding to the 5-6th week of pregnancy, two types of chorionic villi were encountered each having a different structure of the connective tissue stroma: avascular (Fig. 1) and vascular (Fig. 2). In both, the connective tissue stroma has a reticular character; the avascular villi contain somewhat fewer cellular elements while the latter are less polymorphic.

The stroma of the avascular villi can be seen to be a many-branched syncytium of cells having very large nuclei occupying almost the entire cell body. The cytoplasm is clear, faintly basophilic; the nuclei are large, round or oval with evenly distributed clumps of chromatin. The nucleolus stands out distinctly within the nucleus. It is not possible to demonstrate free cellular elements. The matrix of these villi is represented by a thin

network of reticular fibers in the loops of which there lies a finely-grained substance staining bluish with Mallory. The avascular villi possess no collagen fibers that can be demonstrated.

The vascular villi are of two types. The villi stemming from the basic chorionic disc contain more reticular fibers, the villous center only having collagenous replacement. The cellular elements of these villi are represented by fibroblasts forming a syncytium and oriented parallel to the course of the villus. In comparison with the villi of the other type but few free cellular elements can be seen.

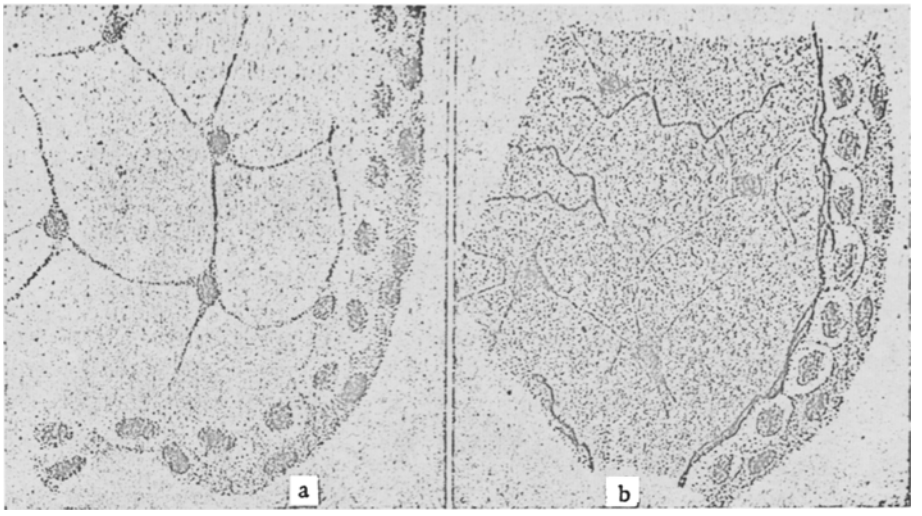

Fig. 1. Avascular chorionic villi (pregnancy 5-6 weeks). Enlargement: ocular 10x, objective 40.
Stain: a) Heidenhain iron hematotoxylin; b) Mallory method.

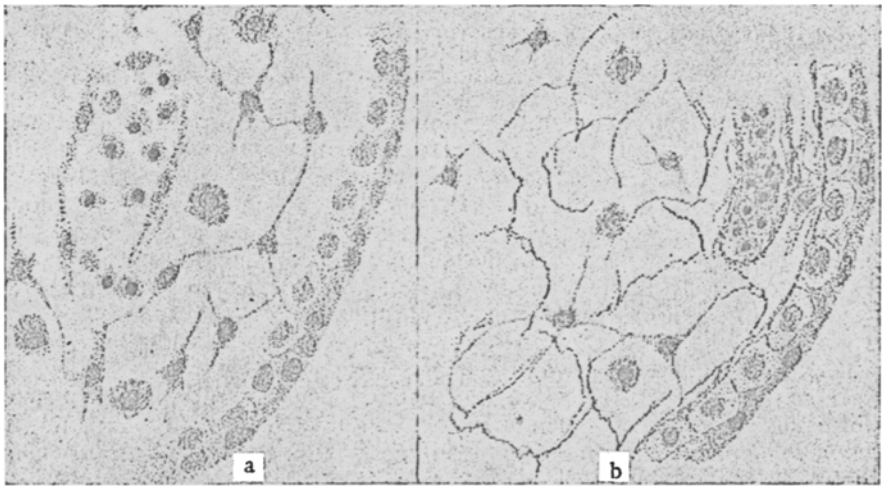

Fig. 2. Vascular chorionic villi (pregnancy 5-6 weeks). Enlargement: ocular 10x, objective 40.
Stain: a) Heidenhain iron hematotoxylin; b) Mallory method.

The stroma of the villi forming terminal branchings previously described has a syncytium of loosely-bound faintly basophilic fibroblasts very reminiscent of the cell elements seen in the avascular villi and has also large individual cells taking the basic stains with greater intensity. The nuclei of these free cells are round or oval, darker, and the clumps of chromatin are larger and less evenly distributed. The cellular cytoplasm appears

porous with uneven edges. These cells are from $1^1/_2$ to twice the size of the preceding cells. Morphologically, these free cells greatly resemble macrophages (histiocytes) seen in the loose connective fibrous tissue (see Fig. 2). The matrix of these terminal villi is represented by a fine net of reticular strands, completely unoriented. Only near vessels and at the boundary with the chorionic epithelium do these fibers form a reticular membrane composed of thicker strands. These villi are bare of collagenous fibers in the stroma. When the structures of the vascular and avascular villi are compared, it can be seen that the avascular type may be regarded as being the more primitive, composed of a syncytium of undifferentiated cells and a fine net of reticular fibers forming the primary villi which either go on and involute or else develop into the secondary type. The secondary villi are more complex, having many branches: at the base, in association with the chorionic plate, they constitute a differentiated connective tissue richly supplied with collagen fibers. The terminal branches are formed of less differentiated tissue rich in reticular strands and cells of the juvenile fibroblastic type, as well as free histiocytic elements.

At all later stages of development of the pregnancy, there are seen villi whose stroma at the base differs from its appearance in the terminal branchings; further descriptions of the connective tissues seen in the villi take this into consideration.

At still later stages of pregnancy, we failed to encounter the primitive avascular villi possessing a connective tissue stroma.

At approximately the stage corresponding to a 7-8-week pregnancy, the terminal branchings of the villi as described above manifest a sharp rise in the number of cellular elements, there being a large increase in free macrophagic cells, usually rounded, with round dark nuclei, porous cytoplasm and uneven edges. Clasmatocytes appear. Occasionally, two-nuclei cells are seen (Fig. 3). The second cell type, as before, is a syncytium of irregular small cells (fibroblasts). Their nuclei are oval or round, with nuclei of a lighter hue than that of the free cellular elements. In the fibroblasts mitoses are seen quite frequently.

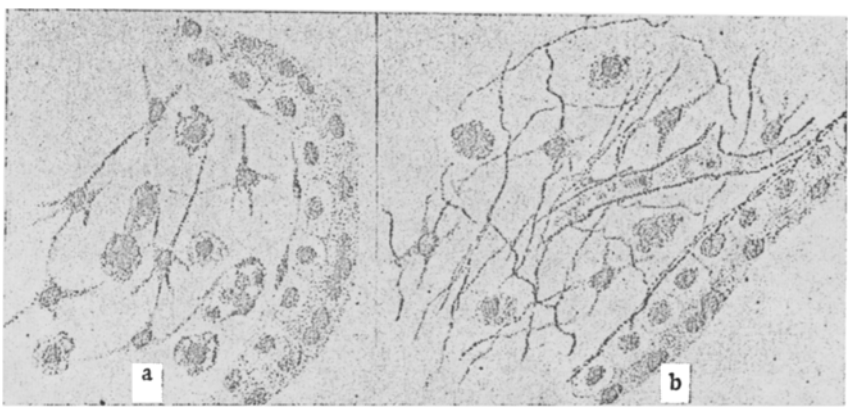

Fig. 3. Terminal chorionic villi (7-8-week pregnancy). Magnification: ocular 10x, objective 40.
Stain: a) Heidenhain iron hematoxylin; b) Mallory method.

At the very base of the villi, in close association with the chorionic plate, the cellular elements are fewer than further in the terminal branchings. Here one encounters principally fibroblasts and fibrocytes oriented along the long axis of the villus. In the central portion of the villus, they are especially numerous. Free, macrophage-type cells are not numerous in the basal divisions of the villi and in their disposition a certain order can be seen: they lie at the periphery of the villus, near the chorionic epithelium. Here in the matrix the collagen, not reticular, fibers predominate lying more frequently towards the center of the villus. In the central portion of the larger villi, as if ensheathed in a case of collagen fibers, lie the larger vessels-arteries and veins. The capillaries locate at the periphery being separated from the other tissues of the villus by a reticular membrane.

At the 9-10, and 12-12-week stages of gestation there is observed some diminution in the cellular elements and among the latter – a relative increase in the fibroblasts when compared with cells of the macrophage type.

The macrophage-type cells cannot be as definitely related to their earlier stages, the cytoplasm being somewhat more vacuolated and clasmatocytosis not being observed. The fibroblasts lose their continuity, amitotic

division becomes more common. In the matrix of the villi, collagenous fibers come to predominate, this being especially noticeable in the larger villi.

At the 15-16-week stage of gestation, the differentiation of the connective tissues is at its sharpest. At the bases of the villi the stroma consists principally of ground substance with a predominance of collagen fibers. Among the cellular elements elongated fibroblasts are most common; outgrowths are few, merging with the fibers of the intermediate substance. The nuclei of the fibroblasts are elongated on the long axis of the cell, becoming almost stick-like and staining rather intensively with the basic dyes. As the branchings of the villi are followed, the structure changes (Fig. 4): in the terminal twigs there is less of the ground substance, principally reticular fibers so that only around the vessels and in the central portions of the villi are collagen fibers to be observed.

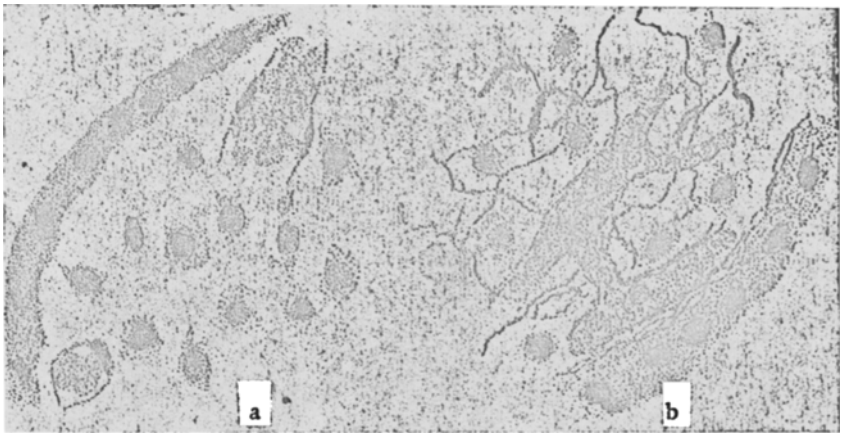

Fig. 4. Terminal chorionic villi (15-16-week pregnancy). Magnification: ocular 10×, objective 40.
Stain: a) Heidenhain iron hematoxylin; b) Mallory method.

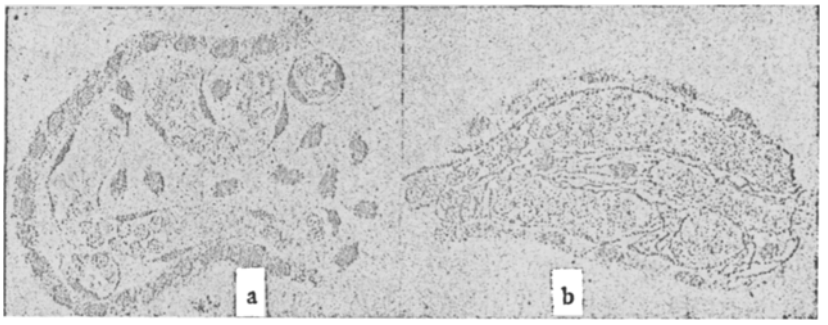

Fig. 5. Terminal chorionic villi (pregnancy 10 lunar months). Magnification: ocular 10×, objective 40.
Stains: a) Heidenhain iron hematoxylin; b) Mallory method.

The cellular elements in the terminal portions of the villi are more varied than they are at the base, the fibroblasts still predominating but differing considerably from the fibroblasts seen at the bases. These are multi-branching cells with oval or round nuclei, manifesting frequent amitoses. The cytoplasm of these fibroblasts stains so poorly that the cell boundaries are hard to define and syncytial-type union among the cells is not to be demonstrated. Free, histiocyte-type cells are here quite few, stain with greatly diminished intensity as compared to the earlier stages; the cytoplasm is markedly vacuolized. At the bases of the villi, as in the earlier stages, lie the larger blood vessels while in the branchings are the capillaries. They lie ensheathed in a fairly heavy layer of collagen (thicker at the villous base).

The structure of the connective tissues in the villi of a mature placenta at birth in principle is no different from the same tissue at the 15-16-week stage (Fig. 5). It is only important to note that, at term, the number of blood vessels increases sharply, the capillaries, in particular, dilating and assuming almost a sinusoidal appearance.

On the basis of the considerations presented above, we can conclude that the connective tissue stroma of the placenta is fully formed by the end of the fourth month; in later stages, because of the intensive growth of the fetus, there is only an increase in the number of blood vessels.

SUMMARY

The author studied the development of the connective tissues in the primary and secondary chorionic villi from the earliest stages to term. He found in the former that the reticular tissue was transformed quite early into loose connective tissue in which collagen fibers came to predominate. The stroma of the terminal villi retained its reticular nature much longer. Up to the sixth week of pregnancy, the terminal villi possess both vascular and avascular types. The development of the connective tissue stroma was complete by the fourth month. Later only the number and caliber of the blood vessels increased.

LITERATURE CITED

[1] V. K. Beletsky, Dissertations of Scientic Research in the Medico-Biological Sciences*, 1949, 7, p. 85.

[2] T. V. Borima. Argyrophilic Content of the Placenta Under Normal and Certain Pathological Conditions,* Dissertation, Kiev, 1951.

[3] T. S. Gerasimenko, Dissertations of Scientic Research in the Medico-Biological Sciences*, 1949, 7, p. 91.

[4] K. P. Ulesko-Stroganova, Akusherstvo i Ginekol., 1895, No. 9, Vol. 4, p. 297.

[5] B. Tenney, Am. J. Obstet. Gynecol., 1935, No. 29, Vol. 6, p. 819.

* In Russian.