LOCAL BLOOD VESSEL FORMATION IN THE HUMAN CHORION IN THE EARLY PERIODS OF NORMAL PREGNANCY

(UDC 611-013.84:611.13/ .16)

A. I. Brusilovskii

Department of Histology and Embryology (Head—Professor B. P. Khvatov), Crimean Medical Institute
(Presented by Active Member of the Academy of Medical Sciences of the USSR . . .*)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 10,
pp. 99-103, October, 1965
Original article submitted April 3, 1964

It is now held that disturbances of the vascular system of the fetal part of the placenta play an important role in the pathogenesis of the prenatal mortality of the fetus and development of pathological pregnancy [1, 8, 9]. The evaluation of the state of the arterio–venous network of the chorion in pathological states must be based on knowledge of the principles governing the formation of the vascular system of the placenta in the conditions of normal pregnancy.

Several workers have studied the local development of blood vessels in certain organs of human and mammalian embryos [10-12, 17-19, and others]. There are reports in the literature of the development of the first vessels in man in the mesenchyme of the extraembryonic portions—in the yolk sac and chorion [13]. However, no description could be found of any special investigations of the local blood vessel formation in the human chorion.

EXPERIMENTAL

Altogether 82 human chorions during the first 3 months of normal pregnancy were studied. Different methods of fixation and embedding of the pieces of chorion in paraffin wax were used. Serial sections were cut to a thickness of 10 μ. The following staining methods were used: Boehmer's, Carazzi's, and Heidenhain's hematoxylins with eosin, and special histochemical methods for the study of polysaccharides (the PAS reaction with controls, toluidine blue, alcian blue, and methylene blue at pH 2.62-7.25, taking into consideration the results of methylation, demethylation, sulfatation, and enzyme controls, and Hale's reaction, phosphate (Gomori's reaction), RNA (Brachet's method), DNA (Feulgen's reaction), lipids (staining with Sudan III, with Nile blue sulfate by Smith's method, and with Sudan black), and iron compounds (Prussian blue and McCallum's reaction).

Graphic reconstructions were used to study the topographic relations between the blood islets in the stroma of the villi. The special features marking the differentiation of the cells taking part in the formation of the local blood vessels in the chorion were studied by B. P. Khvatov's karyometric method [15, 16]. In each case 200 nuclei were drawn from the following groups of cells: the cells of the stroma of the chorion (of the histiocyte type), cells forming blood islets, and cells of the endothelium of the functioning blood vessels. Variance curves were plotted from the results of measurement of these nuclei. The results of statistical treatment of the corresponding variance series were analyzed. The significance of the difference between the arithmetical mean values of the sizes of the cell nuclei was verified by the formula:

$$M_1 - M_2 \geqslant 3\sqrt{m_1^2 + m_2^2}.$$

The volumes of the cell nuclei were calculated from the formula for an ellipsoid [20].

* Name omitted in Russian Original—Publisher's Note.

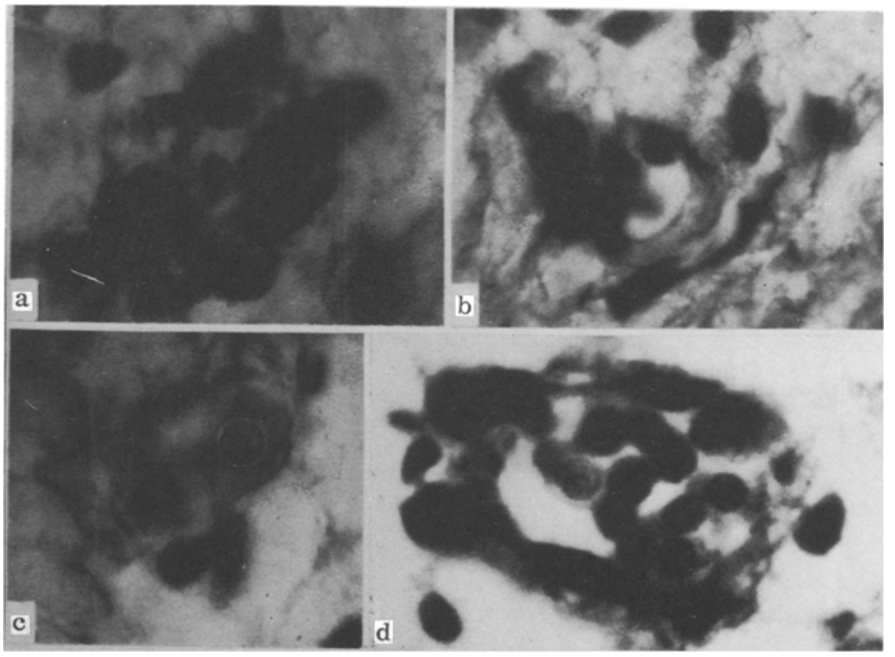

Fig. 1. Successive stages of local blood vessel formation in the stroma of a human chorionic villus. 6-Week pregnancy. a, b) Cluster of cells of histiocyte type; c) appearance of a primary erythroblast; d) a formed blood vessel. Photomicrograph. Hematoxylin–eosin. Objective 100 ×, immersion. Ocular 8 ×.

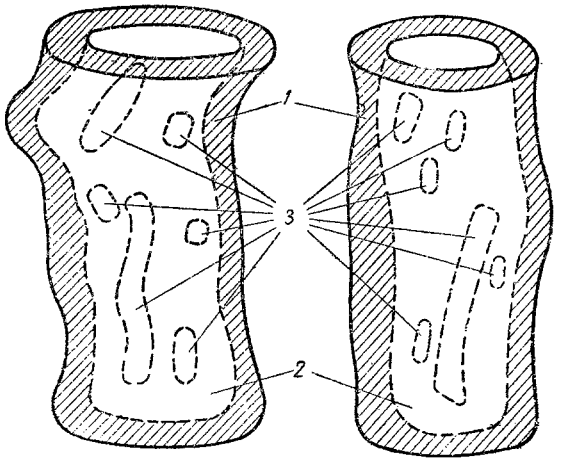

Fig. 2. Distribution of blood islets in the stroma of a villus. Graphic reconstruction of a part of the villus. 1) Epithelium covering the villus; 2) connective tissue stroma of the villus; 3) blood islet. 220 ×.

DISCUSSION OF RESULTS

By the end of the first month of pregnancy intensive processes of differentiation are taking place in the stroma of the villi. These are especially marked in the villi lying in the decidual membrane, presumably in connection with their special functions. As a result of histochemical analysis it was established that the morphological differentiation of the tissue elements is closely related to their histochemical differentiation, which is dependent on the formation of the varied functions of the provisional organ [2, 4].

The differentiation of the cells in the stroma of the villi is characterized by a number of special features. In the early stages of ontogenesis the chorionic villi contain fibroblasts and round cells of the histiocyte type. With age, the fibroblasts, which are normally joined together by their processes, lose these links. In the stroma of the mature chorion fibrocytes can be distinguished. In the villi of different caliber most cells are in the smaller ramifications. The number of cells of histiocyte type diminishes towards the end of the 3rd month of pregnancy. The changes in the dimensions of the nuclei of the connective tissue cells in the stroma of the chorion agree with the dynamics of the nucleic acids and reflect the functional changes in the villi at different periods of pregnancy [3, 5, 6].

In some parts of the stroma of the villi the local formation of blood vessels can be observed. This begins with the formation of a blood islet. Cells of histiocyte type, with round or slightly oval, basophilic nuclei, are grouped together in the connective tissue of the villi, their number varying from 6-8 to 12-14. Subsequently the outermost

TABLE. 1. Karyometric Characteristics of Size of Cell Nuclei of the Connective Tissue Stroma of the Chorion and of the Endothelium of Functioning Blood Vessels of the Villi

Nucleus of cells	Volume		Mean diameter		Smaller diameter		Larger diameter	
	$M \pm m$	σ	$M \pm m$	σ	$M \pm m$	σ	$M \pm m$	σ
Of histiocyte type in connective tissue stroma of chorion	1 344 ± 30,32	± 470	13,78 ± 0,10	± 1,43	11,30 ± 0,09	± 1,30	16,18 ± 0,16	± 2,27
Of blood islet of connective tissue stroma of villi	1 117 ± 22,4	± 315	12,83 ± 0,09	± 1,30	11,01 ± 0,09	± 1,34	14,60 ± 0,15	± 2,06
Of endothelium of functioning blood vessels of the human chorion	1 302 ± 28,8	± 406	13,93 ± 0,09	± 1,24	8,93 ± 0,08	± 1,18	19,15 ± 0,16	± 2,27

Notes. 1. The karyometric characteristics of the sizes of the cell nuclei are given in conventional units (1 conventional unit = 0.416 μ). 2. The data presented here are those of karyometry and also of graphic reconstruction obtained in a 7-week chorion (in the remaining objects they were similar).

cells of the group and their nuclei become longer and form the vessel wall. The centrally situated cells of the group become more oxyphilic and give rise to blood cells. These are primary erythroblasts, round oxyphilic cells with nuclei in which karyoplasmic structures can be distinctly seen, and they are surrounded by basophilic histiocytes (Fig. 1).

To determine the topographic relations between the blood islets in the stroma of the villi, a graphic reconstruction was made of certain areas of the villi (Fig. 2). These investigations showed that the blood islet occupies a larger or smaller area in the chorionic villus. In many villi several such islets are seen, one above the other, but separated by areas of extraembryoblastic connective tissue. In the later stages they evidently join together to form blood vessels.

It is interesting that, according to these findings, the local blood vessel formation persists longer in the smaller villi, i.e., in those further from the chorionic plate. In the latter and in the larger villi, which are invaded immediately by blood vessels from the allantois, fewer blood islets are seen. In the small ramifications of the villi the ratio between the number of functioning blood vessels and the number of them formed locally averages 1:0.9.

Karyometric investigations confirmed the distinctive cytological changes in the cells and their nuclei in the course of differentiation. The mean diameter of the nucleus of the cells of histiocyte type in the stroma of the villi is 13.78 conventional units. When these cells form a blood islet, the diameter of their nucleus falls to 12.83 conventional units. Similar changes take place in the volume of these nuclei. Our findings also show that the nuclei of the endothelial cells of the functioning blood vessels are most elongated (the ratio between two mutually perpendicular diameters is 0.47:1), while the nuclei of the cells of the blood islet are almost round (the ratio between the diameters is 0.76:1).

The full karyometric characteristics of the investigated nuclei are given in Table 1.

When describing the special properties of the vascular endothelium of the placenta, A. Ya. Sul'din (1963) also pointed out that the endothelial cells of the chorion and their nuclei are the most elongated.

The results of this special investigation of the formation of the blood islets thus confirm the important role of local blood vessel formation in the development of the unified arterio–venous system of the chorion. It may be concluded that the vessels of the placenta are formed both by invasion of vessels from the allantois and by the union of locally formed capillaries with the main trunks [7]. These findings must be taken into consideration when the causes of pathological embryogenesis are being evaluated.

LITERATURE CITED

1. V. I. Bodyazhina, Akush. i gin., 2 (1963), p. 3.
2. A. I. Brusilovskii, Trudy Krymsk. med inst. 32 (1961), p.33.
3. A. I. Brusilovskii, Abstracts of Proceedings of Symposia of the All-Union Scientific Society of Anatomists, Histologists, and Embryologists [in Russian], Moscow (1962), p. 62.
4. A. I. Brusilovskii, Akush. i gin., 2 (1963), p. 10.
5. A. I. Brusilovskii, Theses of Lectures on the Embryonic Development of Animals and Man [in Russian], Leningrad (1963), p. 28.
6. A. I. Brusilovskii, Byull. éksper. biol., 7 (1963), p. 29.
7. A. I. Brusilovskii, Abstracts of Proceedings of the 11th All-Union Congress of Obstetricians and Gynecologists [in Russian], Moscow (1963), p. 76.
8. A. S. Egorov, Akush. i gin., 4 (1962), p. 52.
9. J. Shordania (I. F. Zhordania), Arch. Gynäk. 135 (1929), p. 568.
10. V. P. Zhuk, Doklady Akad. Nauk SSSR,107 1 (1956) p. 167.
11. V. P. Zhuk, Data on the comparative morphology of the connective tissue of the chorion in man and certain mammals. Candidate dissertation, Novosibirsk, (1957).
12. N. D. Zaitsev, Abstracts of Proceedings of the 6th All-Union Congress of Anatomist, Histologists, and Embryologists [in Russian], Khar'kov (1958), p. 462.
13. A. G. Knorre, Texbook of Obstetrics and Gynecology [in Russian], 2 book 1 Moscow (1963), p. 9.
14. A. Ya. Sul'din, Folia histochem. cytochem. 1 Supple. 1 (1963), p. 137.
15. B. P. Khvatov, Labor praktika, 9 (1932), p. 2.
16. B. P. Khvatov, Biol. zh. 11, 4-5 (1933), p. 343.

17. Yu. N. Shapovalov, Formation of the mesenchyme and development of the blood islets and blood vessels in mammals. Candidate dissertation, Simferopol', (1956).
18. Yu. N. Shapovalov, Trudy Krymsk. med. inst. 30 (1961), p. 13.
19. I. M. Yarovaya, Trudy Krymsk. med. inst. 30 (1961), p. 161.
20 J. P. Schade, and A. V. Harreveld, J. comp. Neurol., 117 (1961), p. 387.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
