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Blood Ultrastructure in Abnormal Pregnancy

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Electron microscopic examination of peripheral blood of women with placental failure of different origin showed specific features of cells and noncellular components: high contact activity, phagocytic activity, highly active B-lymphocytes (plasma cells), cytolysis, cytoplasm clasmatosis, small segmentation of neutrophil nuclei, high concentration of noncellular components presenting as ultramicrogranules and fibrils, cell fragments, membranes, and granules, and atypical blood components (fibrinoid structures, bacteria, and virus-like particles). These data can be used for selecting optimal simple tests for diagnosis and treatment monitoring.

Key Words: *pregnancy; disease; blood; ultrastructure*

In this study we proceeded with electron microscopic analysis of structural components of peripheral blood (PB) of women with pathological pregnancy consisting in placental failure [11]. Previously we demonstrated the advantages of ultrastructural analysis of PB components and the possibility of parallel assessment of the function of effector elements of many regulatory systems in a single preparation [2-6]. High sensitivity of electron microscopic analysis permits its use for study of the slightest borderline deviations from the norm at the early stages of disease. Ultrastructural analysis of blood components demonstrates a great variety of elements, making possible the differentiation of pathogenetic factors.

Using electron microscopy, we analyzed structural elements of PB in women with placental failure of different etiology for detecting changes pathogenetically important for circulatory and endothelial disorders in blood vessels.

MATERIALS AND METHODS

Eighty-eight women were examined during the first, second, and third gestation trimesters. Ten of them were controls with normal course of pregnancy and normal delivery without complications, other women were at risk of placental failure: 29 women with first pregnancy living in an ecologically unfavorable region (Archangelsk), 10 of these with gestation hypertension; 20 women at high risk of placental failure (no complications in 16, hypertension in 4); and 29 women with habitual miscarriages. Material for electron microscopy was processed and analyzed as described previously [3-5]. Blood components were fixed in glutar aldehyde and osmium tetroxide in phosphate buffer, ultrathin sections were contrasted with uranyl acetate and lead citrate [4,5].

RESULTS

Electron microscopy of plasma membranes, cytoplasmic matrix, nuclei, cell organelles, and noncellular components of PB was carried out during normal pregnancy and pregnancy complicated by placental failure. Platelets and neutrophils showed the highest variability of the plasma membrane and cytoplasmic organelles. Erythrocytes differed only by the intensity of contact interactions with the adjacent cells, the ultrastructural density of the matrix was virtually not changed, and hemolysis was extremely rare. Lymphocytes and monocytes differed mainly by

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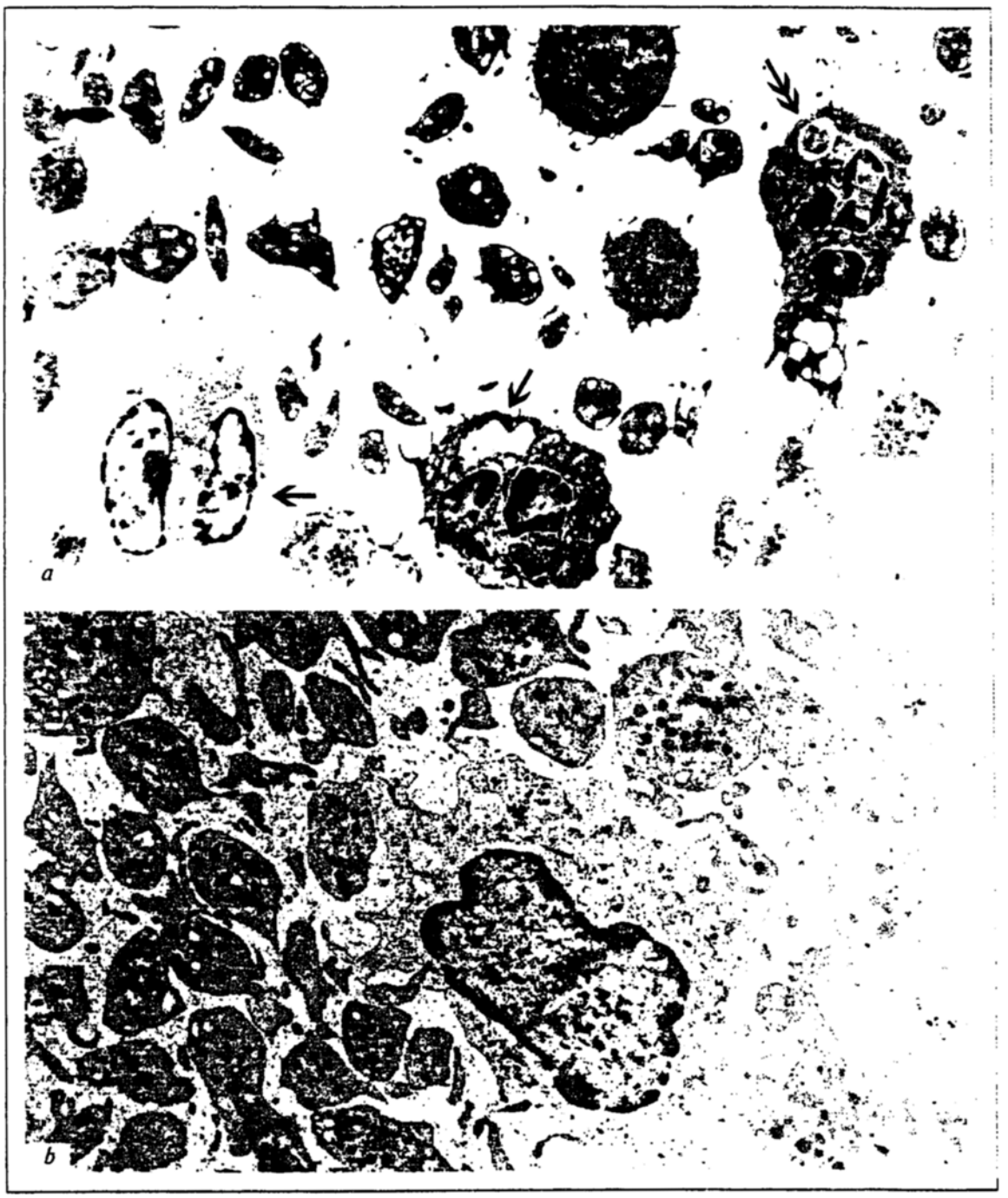

Fig. 1. Activated neutrophils, monocytes, lymphocytes, and platelets with a high density of noncellular components in a patient at a high risk of placental failure during 34th week (*a*) and in a woman living in an ecologically unfavorable region during the 29th week (*b*). ×8000. *a*) Platelet phagocytosis by a neutrophil (double arrow), large phagosomes in neutrophil and monocyte (arrows); high concentration of noncellular components: ultramicrofibrils, granules, membranes, and fragments between platelets and leukocytes. *b*) Monocyte in a state of active phagocytosis surrounded by activated platelets; high concentration of ultramicrofibrils, granules, and fragments between these platelets.

intensity of formation of processes and by the set of organelles in the cytoplasm and nucleus.

Normally, the majority (>75%) of the studied PB components are in the state of minimal structural and functional activation; this level is used in comparative analysis. Less than 25% structural components were activated, which is due to normal cycle of cell activity fluctuations. Increased activity of blood elements from the first to third trimester of normal pregnancy was within the same range.

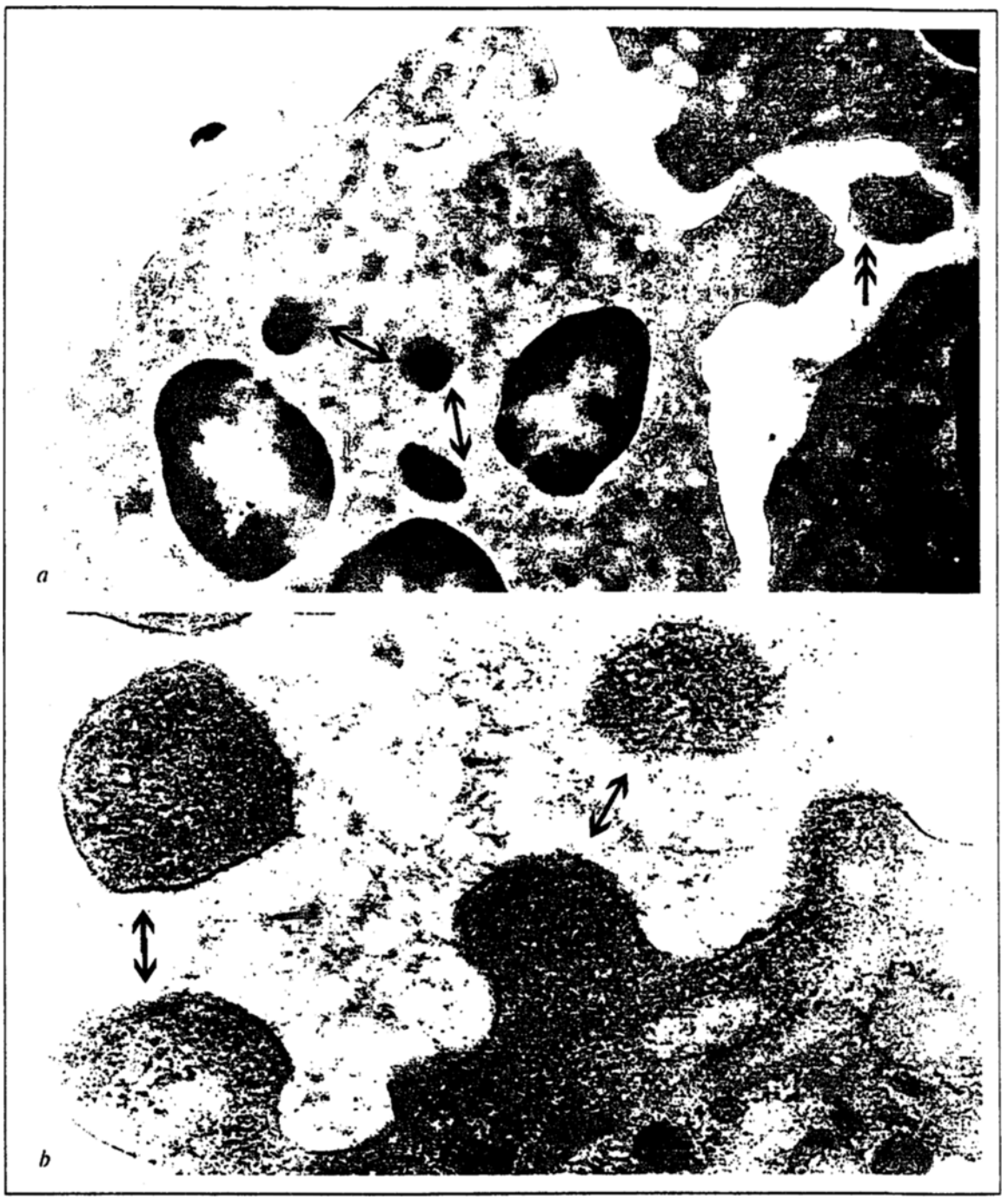

Fig. 2. Small segmentation of the nucleus and cytoplasm clasmatosis in neutrophils in women from an ecologically unfavorable region during weeks 20 (*a*) and 31 (*b*) of pregnancy. *a*) Segmentation of nucleus (arrows) and cytoplasm clasmatosis (double arrow); high concentration of ultramicrofibrils and cell fragments. ×16 000. *b*) Neutrophil cytoplasm clasmatosis (arrows); high concentration of ultramicrofibrils. ×40 000.

In placental failure, PB components are in a state of increased (more than 50%) and high (75%) activation. Noncellular components (ultramicrofibrils and ultramicrogranules forming floccular formations that join surface elements of blood cell membranes and not differing from cell glycocalix) were often seen between blood cells. In some, women non-cellular macromolecular complexes were of high density. There were fragments of cell membranes (possessing thromboplastic activity [5,8]), fragments of cell cytoplasm (particularly near neutrophils, monocytes, lymphocytes, and platelets), cell granules released from cells either during secretory activity or after cell destruction, and fibrinoid structures (Figs.

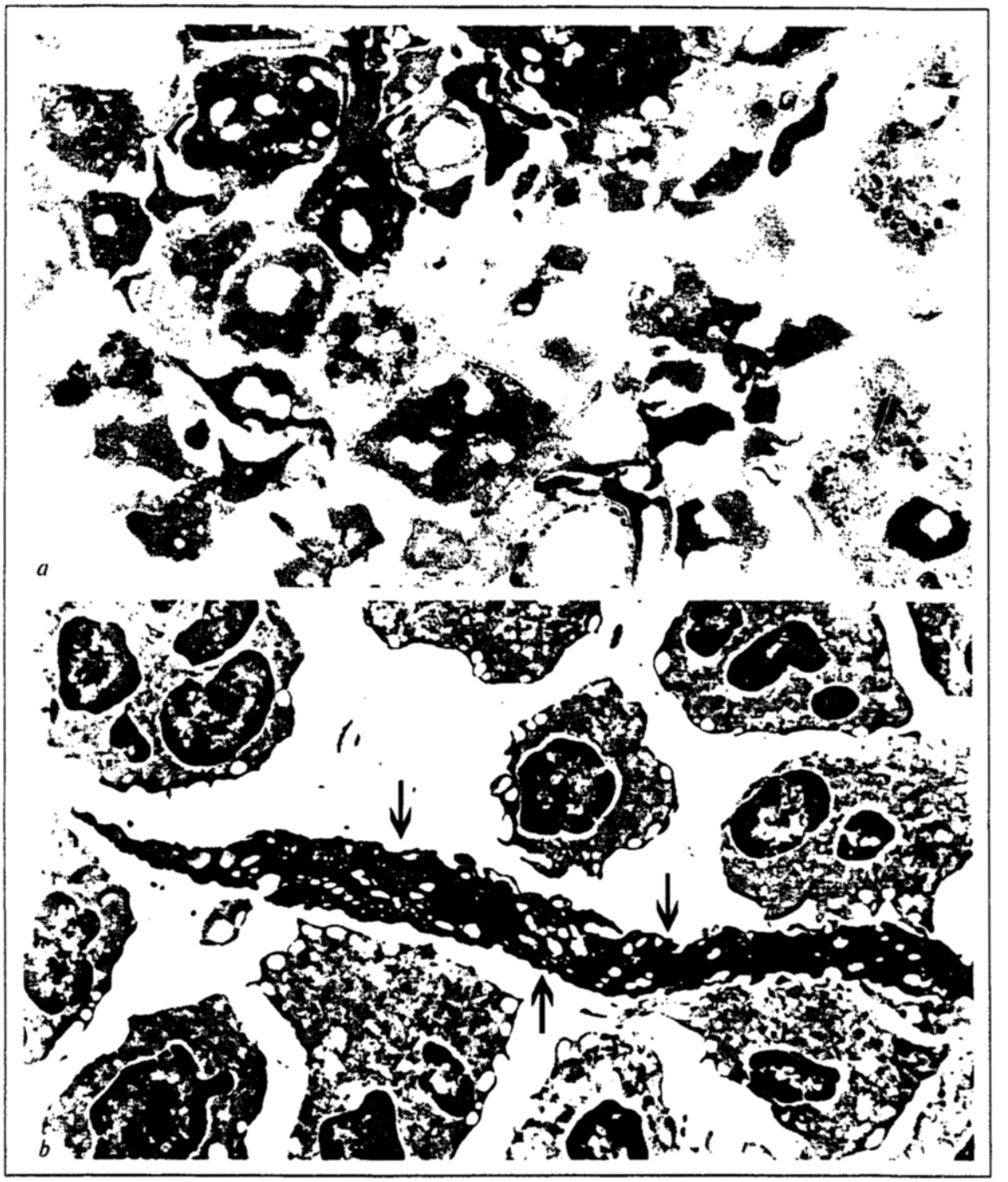

Fig. 3. High contact and phagocytic activity of peripheral blood components in a patient at a high risk of placental failure during week 40 (*a*) and a patient with habitual miscarriages during week 20 (*b*). *a*) In the center: cell fragments and vacuolized platelets; at the periphery: activated neutrophils. ×12 000. *b*) A large fibrinoid structure (arrows) surrounded by activated neutrophils. ×8000.

1-3). In few women, rudiments of microorganisms, probably in a state of destruction, and virus-like particles were detected.

Minimal expression of structural and functional activation of the studied blood components consisted in the following: blood cells (erythrocytes, platelets, neutrophils, lymphocytes, and monocytes) had intact plasmalemma, few cell-to-cell contacts, and a minimal set of organelles with signs of functional activation (production of nucleic acids, protein, and lipids).

Increased structural and functional activation was characterized by a notable prolongation of short and long cell-to-cell contacts, cytoplasmic processes,

TABLE 1. Ultrastructural Characteristics of PB Components in Women with Placental Failure of Different Etiology

Ultrastructural features	Pathology			
	ecologically unfavorable region (*n*=29)	arterial hypertension (*n*=10)	high risk of placental failure (*n*=20)	habitual miscarriage (*n*=29)
High contact activity of components	+	+	++	+++
Phagocytosis of components	+	+	++	+++
Highly activated B-lymphocytes	+	+	++	+++
Cytolysis	+	—	—	+
Clasmatosis of cytoplasm	++	+	+	++
Small segmentation of nuclei	+	—	—	—
High concentration of noncellular components	+	+	++	+++
Fibrinoid structures	+	+	+	+
Bacteria outside and inside cells	+	+	++	+++
Virus-like particles	—	—	—	+

Note. —/+++: relative incidence and degree of ultrastructural signs expression; — not observed.

and increased number of activated cytoplasmatic and nuclear ultrastructural complexes and organelles.

Activated PB cells were characterized by a greater number of extensive close cell-to-cell (mainly erythrocytes and platelets) contacts, high activity of nuclear (diffuse chromatin and nucleoli, particularly in lymphocytes and monocytes) and cytoplasmatic structures (by the number of mitochondria, ribosomes, elements of cytoplasmatic reticulum, activated specific granules, lysosomes, phagosomes, and vacuoles in platelets, neutrophils, lymphocytes, and monocytes).

However the status not only of cells, but also of noncellular components of the plasma, such as cell fragments, granules, membranes, and macromolecular complexes (ultramicrogranules and fibrils) is important, namely, their number and composition. The density of these elements is high in the blood of women with placental insufficiency of different etiology; their blood contains such atypical elements as fibrinoid formations, bacteria, and virus-like particles.

The status of PB components in normal pregnancy is considered as a threshold reference value (+) for detecting activation of these components. The level with at least 50% (++) of activated components is considered as increased activation. High activation is a value of at least 75% when sometimes more than 25% of blood cells are in state of high activation (+++).

Multifactorial qualitative ultrastructural analysis of PB components in women with placental failure showed that the pathological process promotes accumulation of abnormal ultrastructural signs from the first to the third trimester of pregnancy. Specifically, close contacts are intensely formed between cells (primarily erythrocytes and platelets), blood cells, and activated cell fragments are accumulated (Figs. 1-3).

These signs point to pathogenetic mechanisms leading to the development of placental failure (Table 1), which is a specific phenomenon associated with various complications of gestation. The first sign is high contact activity of blood components. This activity can be caused by fetal antigens (penetrating in maternal vascular bed through the placenta as macromolecular structures and platelets, erythrocytes, etc.) [7-10] and by microorganisms (bacteria and viruses) persisting in maternal organism for a long time [1]. Electron microscopy shows the formation of contact aggregations and agglomerates activated in the presence of antigens or antigen-antibody complexes adsorbed on cells (Fig. 1, *b;* 3, *a, b*), macromolecular complexes, and cell fragments (Fig. 1, *a, b;* 2, *a, b*).

Female organism reacts to antigenic components by activation of lymphocytes, specifically, of B cells producing antibodies and by phagocytosis of components, including those of fetal origin, by monocytes and neutrophils (Fig. 1, *a, b*). Environmental factors can cause cytoplasm fragmentation and small segmentation of the nucleus, for example in neutrophils (Fig. 2, *a, b*).

It should be emphasized that we selected the most distinctive signs from numerous ultrastructural features; such signs are never observed in health, and a comparative differential analysis of placental failure of different etiology by these signs was carried out

(Table 1). Our study confirmed that the incidence of abnormal signs increases in women with grave clinical manifestations of placental failure (Table 1).

REFERENCES

1. A. S. Ankirskaya, *Akush. Ginekol.*, No. 6, 13-16 (1995).
2. G. U. Asymbekova, P. D. Bonartsev, T. B. Ochan, *et al.*, *Eksper. Klin. Farmakol.*, **58**, No. 2, 35-39 (1995).
3. P. D. Bonartsev, in: *Prevention, Diagnosis, and Treatment of Women with Habitual Abortions and Care of Their Children* [in Russian], Moscow (1990), Part 1, pp. 113-119.
4. P. D. Bonartsev and E. M. Demidova, *Akush. Ginekol.*, No. 10, 15-18 (1987).
5. P. D. Bonartsev and E. M. Demidova, *Vestn. Akad. M. Nauk SSSR*, No. 5, 23-25 (1990).
6. E. M. Vikhlyaeva, O. M. Supryaga, V. A. Burlev, and P. D. Bonartsev, *Ekologiya Cheloveka*, No.4, 24-28 (1996).
7. E. D. Zagorodnyaya, N. G. Budazhabon, L. A. Baklitskaya, and L. G. Erofeeva, *Akush. Ginekol.*, No. 10, 46-49 (1987).
8. B. I. Kuznik, N. B. Vasil'ev, and N. N. Tsybikov, *Immunogenesis, Hemostasis, and Nonspecific Resistance of an Organism* [in Russian], Moscow (1989).
9. V. I. Kulakov, L. E. Murashko, and V. A. Burlev, *Akush. Ginekol.*, No. 6, 3-5 (1996).
10. A. Ya. Kul'berg, K. V. Voronin, N. V. Kryachkova, *et al.*, *Immunologiya*, No. 3, 70-72 (1991).
11. G. M. Savel'eva, M. V. Fedorova, P. A. Klimenko, and L. G. Sigalova, *Placental Failure* [in Russian], Moscow (1991).

Morphological Assessment of Growth Capacity of the Central Nervous System Axons in a Peripheral Nerve

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Embryonal rudiments of rat neocortex and spinal cord survive for 60 days after subperineural transplantation into the distal part of crossed sciatic nerve in adult animals. Embryonal cell elements are differentiated from neuroepithelial cells and neuroblasts to mature neuro- and gliocytes. Transplanted spinal neuron axons migrate from the transplant and are myelinized with recipient peripheral nerve Schwann cells. A cavity lined with ependyma-like cells is often formed at the site of dying grafted cell elements.

Key Words: *transplantation; nerve tissue; peripheral nerve; regeneration*

Limited reparative regeneration of central nervous system (CNS) tissues prompts the search for new methods correcting and compensating for the lost nervous functions. Peripheral nerve lemmocytes can be employed as a conducting path for CNS axon growth. Schwann cells [7] or fragments of nerve stems of the peripheral nervous system [8,9,11-13] are transplanted into different compartments of damaged brain with this aim in view. We transplanted embryonal rudimental brain into crossed sciatic nerve of adult rats in order to investigate the relationships between CNS neurons and peripheral glia.

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MATERIALS AND METHODS

Fifty male and ten female Wistar rats weighing 200-250 g were used. The sciatic nerve was crossed under ether narcosis and the proximal and distal ends were ligated. Embryonal material was introduced under the perineurium of the distal part of a large nerve stem. Fourteen-day-old Wistar rat embryos were donors, from which brain sites with rudimental neocortex and the spinal cord were isolated. The animals were killed by ethyl ether overdose 7, 14, 30, and 60 days after transplantation. For histological studies, sciatic nerves were fixed in Bouin's fluid. Paraffin sections (5-7 μm) were stained with hematoxylin and eosin and toluidine blue by the method of Niessle. Nerve fibers were

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