cells. This confirms the hypothesis that mucin and pepsinogen can be produced by the same cell [1,4,5].

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Pathomorphology of Rat Lungs During the Postirradiation Period

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Electron microscopy of rat lungs 14 and 30 days after total γ-irradiation in a dose of 2 Gy reveals severe damage to type II pneumocytes and lamellar bodies. These changes coincide with postirradiation pneumonitis and the initial manifestations of focal pneumofibrosis.

Key Words: *irradiation; type II pneumocytes*

It is important to investigate the effect of ionizing radiation on the internal organs and subcellular structures. The lung is a target organ of ionizing radiation that causes radiation pneumonia, fibrous alveolitis, and secondary pneumonia in patients with acute radiation disease [5]. All these respiratory disorders result from alterations of the surfactant (SF) metabolism. Previously, we showed that total irradiation induces disturbances in the metabolism of lung lipids which manifest themselves as an increase in the content of lipid peroxidation products and a decrease in antioxidant activity [6]. However, we did not study structural modifications occurring in SF-producing cells, type II pneumocytes.

In the present study we analyzed pathomorphological alterations induced by ionizing radiation in the lungs at the early stages of postirradiation period.

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MATERIALS AND METHODS

Experiments were performed on adult male Wistar rats (200±10 g) according to the "Regulations on Animal Experiments" of Russian Health Ministry. The rats were γ-irradiated (^{60}Co) in a PX-γ-30 installation with a single dose of 2 Gy at a power of 2.25 Gy/min. Intact rats served as the control. The animals were sacrificed 14 (group 1) and 30 days (group 2) after intraperitoneal injection of hexenal (1.5 mg/100 g body weight).

Lung specimens were fixed in 10% Lilli's neutral formalin, dehydrated in ascending concentrations of ethanol, and embedded in paraffin. Deparaffined sections were stained with hematoxylin and eosin.

For electron microcopy lung pieces (1 mm^3) were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2-7.4), postfixed with 1% osmium tetroxide on 0.1 M cacodylate buffer, dehydrated in ethanols and acetone, and embedded in Epon-Araldite. Semithin sections (1 μ) were cut in an LKB-U ultramicrotome, stained with azure blue II—fuchsin, and viewed under a light microscope. Ultrathin sections were stained with uranyl acetate

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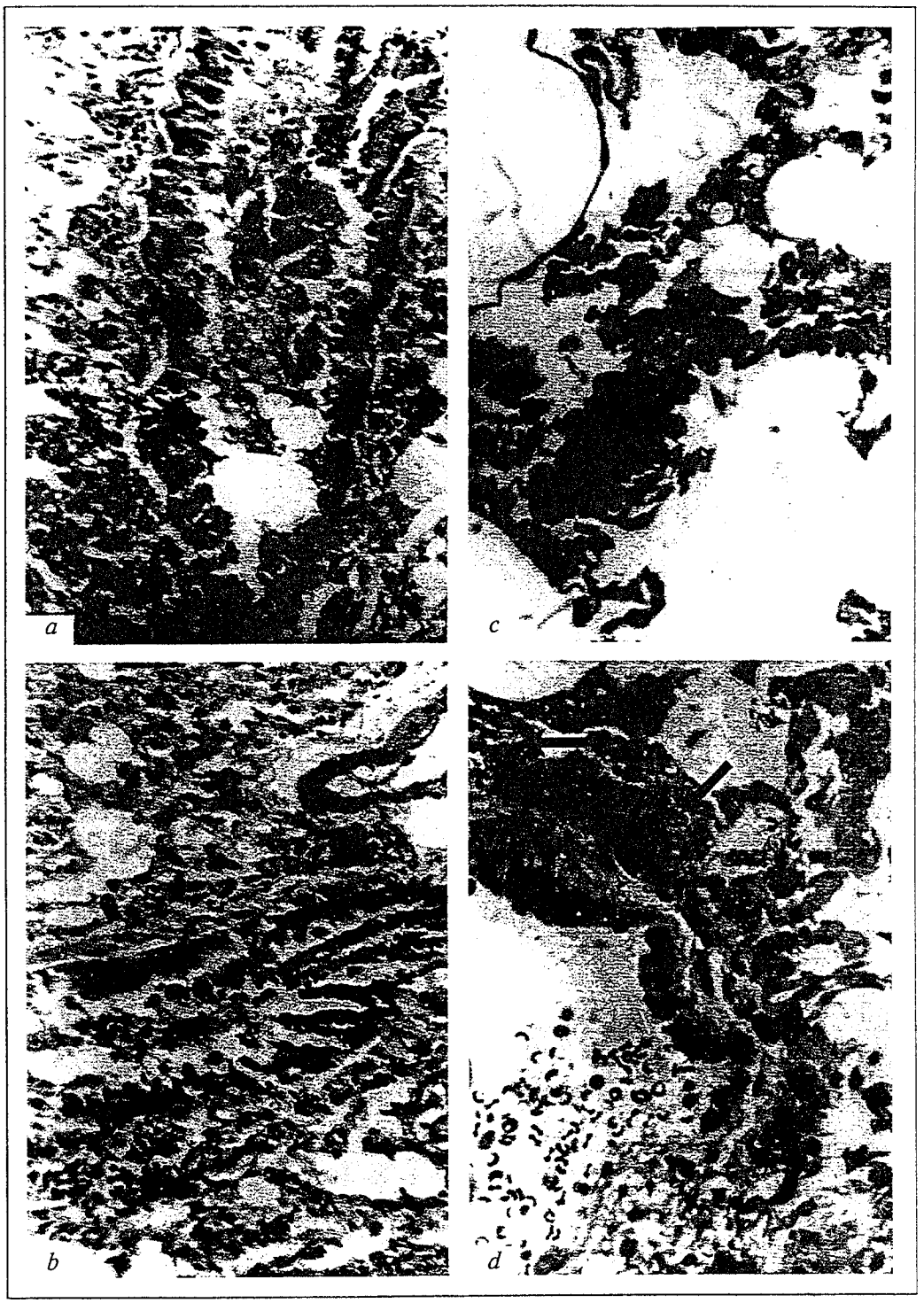

Fig. 1. Pathohistological changes in rat lungs during postirradiation period. *a*) focal dystrophy of bronchial epithelium; note the arrangement of nuclei, desquamation of epithelial cells, and accumulation of secretory product in the lumen; *b*) epithelial dystrophy, desquamation of epitheliocytes, and infiltration of interalveolar septae with mononuclear cells; *c*) dystelectasis and emphysema; *d*) accumulation of mucus, epitheliocytes, and endotheliocytes in the bronchus; "foam" cells are seen in the adjacent alveoli. *a*, *b*) staining with hematoxylin and eosin, magnification 200; *c*, *d*) semithin sections, staining with methylene blue—azure II—fuchsin, magnification 320.

in 8% neutral formalin, rinsed with 0.02 M NaOH, and contrasted in alkaline solution of lead citrate. The preparations were examined in a JEM-100S electron microscope.

RESULTS

Morphological changes typical of pneumonitis or alveolitis were revealed in the lungs of irradiated rats

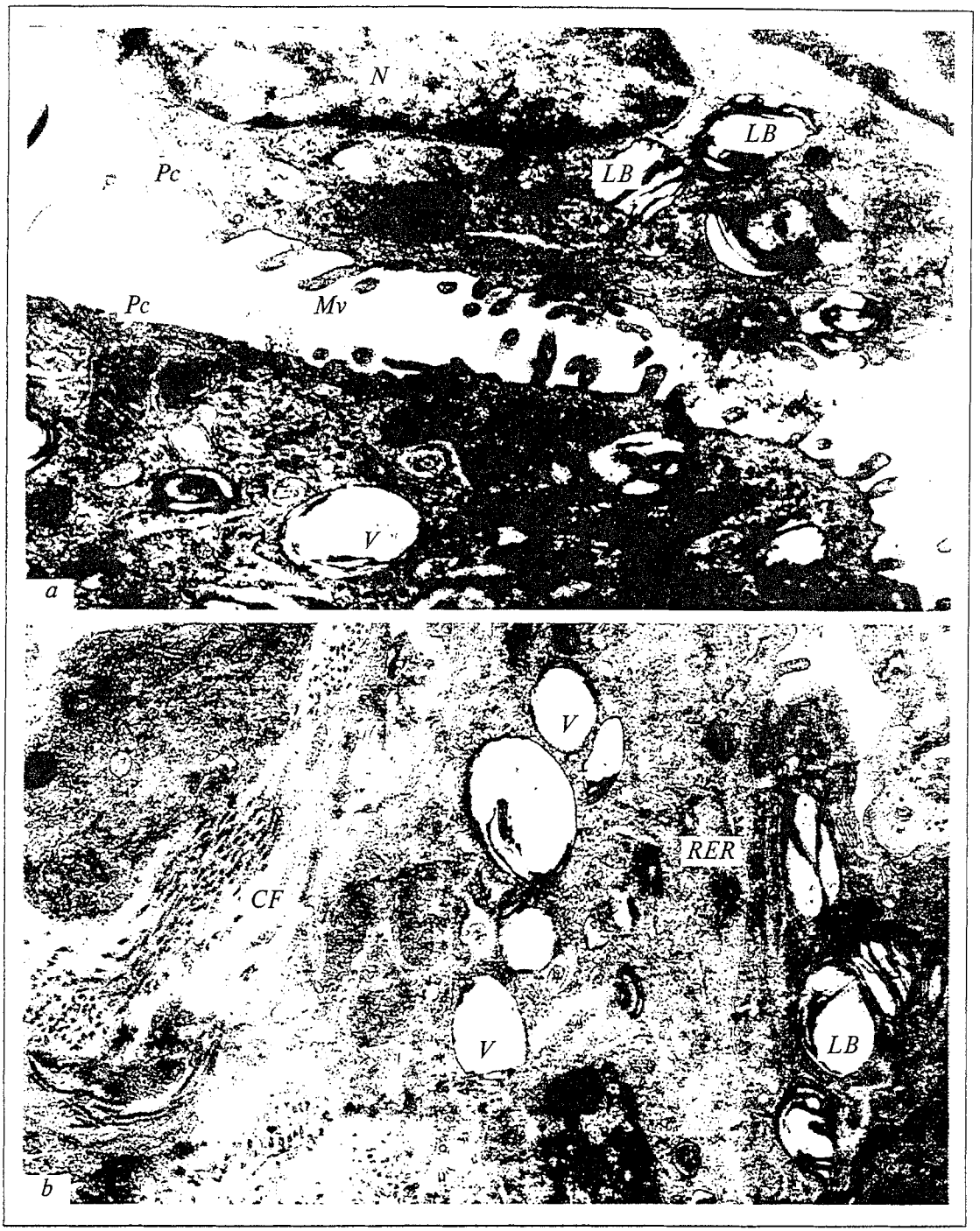

Fig. 2. Electron micrograph of rat lungs after total irradiation. Magnification 15,000. *a*) two type II pneumocytes (*Pc*) with fragments of microvilli (*Mv*), destructive changes in the lamellar bodies (*LB*), and large vacuoles (*V*); *N*) nucleus; *b*) type II pneumocyte with polymorphous vacuoles (*V*) in the cytoplasm; some vacuoles contain fragments of osmiophilic lamellae, the amount of ribosomes in the rough endoplasmic reticulum (*RER*) is decreased, and collagen fibers (*CF*) accumulate around the pneumocyte.

[1,4,5]. Visual examination revealed no changes in the lungs of intact rats. Microscopically, typical structure was preserved; some interalveolar septae were slightly thinned due to outgrowth of the connective tissue.

On day 14 after irradiation, the lungs were edematous and plethoric; small hemorrhagic foci were observed. Sometimes hemorrhagic foci occupied considerable part of lung lobes. Light microscopy revealed pronounced plethora, serous exudate in the bronchi and alveoli, and edema of interalveolar septae. Sometimes diapedesis of erythrocytes was observed. Dystrophic changes in the endothelium and its desquamation occurred in the bronchi (Fig. 1, *a*). The

thickening and infiltration of interalveolar septae by lymphocytes and macrophages without distinct reaction of polymorphonuclear leukocytes proceeded against the background of vascular alterations (Fig. 1, *b*).

Dystelectasis and focal lymphoid-macrophagal infiltration developed by the 30th day of postirradiation period. Dystelectasis was accompanied by emphysema with pronounced polymorphism of terminal alveoli and bronchioli and thinning and disruption of interalveolar septae (Fig. 1, *c*). The fibroblast reaction with focal thickening of interalveolar septae was more pronounced compared with intact animals. Dystrophic and necrotic changes occurred in bronchial epithelium in line with accumulation of eosinophils in the bronchi, which was probably associated with impaired drainage function. Bronchial walls were thickened; sometimes peribronchial tissue was infiltrated by lymphocytes and macrophages. Generally, the infiltrates did not form deep in the lungs and could be easily distinguished from lymphatic follicles located under the epithelium in loose connective tissue of the bronchi.

Numerous large cells with a clear cytoplasm were seen in the alveolar wall on semithin sections (Fig. 1, *d*). It is likely that these cells are the "foam cells" appearing in radiation alveolitis [5]. Aggregation of erythrocytes was observed in the microcirculatory bed.

Electron microscopic study of lungs on day 30 after irradiation revealed pronounced ultrastructural changes in lung tissue and severely damaged type II pneumocytes. Some pneumocytes lost microvilli, which coincided with partial degranulation of the rough endoplasmic reticulum and a decrease in the amount of ribosomes. Subtotal and total destruction of the osmiophilic compound was observed in the lamellar bodies. A considerable proportion of lamellar bodies transformed into large vacuoles with a monolayer membrane (Fig. 2, *a*). In some lamellar bodies, the membranes lost parallel orientation, underwent fragmentation and degradation, and looked like irregular conglomerates of high electron density. Sometimes, extrusion of damaged type II pneumocytes into alveole was observed. In some type II pneumocytes, lamellar bodies were located near the plasma membrane, and their contents was released into alveolar lumen, indicating partial preservation of secretory activity of these cells.

Comparison of semithin sections and electron micrograms showed that vacuolized type II pneumocytes with damaged lamellar bodies are identical to foam cells with clear cytoplasm identified under a light microscope.

Electron microscopy also revealed structural alterations in the blood-gas barrier due to outgrowth of histiocytes and fibroblasts and increased collagen deposition in interalveolar septae. The alveolar-capillary membrane was thickened, and its fibrillar structure was impaired. In the interalveolar septa, oval cells with round nuclei predominated over fibroblasts and polymorphonuclear leukocytes. Loose collagen bundles were seen in the intercellular space (Fig. 2, *b*).

Thus, morphological investigation confirmed the development of postirradiation pneumonitis with the initial manifestations of focal pneumofibrosis and severe changes in type II pneumocytes. The nonspecific response to ionizing radiation manifested itself as a weak productive inflammation.

The following tentative scheme for the development of postirradiation lung syndrome can be proposed on the basis of our findings. Ionizing radiation affects the membranes of type II pneumocytes, which leads to a decrease in surfactant secretion and surface tension, thus impairing lung expansion and contraction. Hypoxia associated with insufficient secretory activity of type II pneumocytes induces pneumonitis resolving in pneumofibrosis. The fibroblast reaction and increased collagen production against the background of hypoxia affect the blood-gas barrier, which aggravates respiratory insufficiency and hypoxia, thus promoting the progression of pneumofibrosis. A vicious circle formed as a result of these perturbations is responsible for the remote postirradiation consequences, namely, diffuse pneumosclerosis that develops within at least six months after total irradiation [2-5].

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