# ELECTRON-MICROSCOPIC AND AUTORADIOGRAPHIC STUDY OF THE EFFECT OF A HELIUM-NEON LASER ON CHRONIC INFLAMMATORY CHANGES IN THE BRONCHI

L. M. Nepomnyashchikh, V. V. Polosukhin,
G. I. Nepomnyashchikh, and V. P. Tumanov

UDC 616.233-002.2-085.849.19-036.8-087.4

KEY WORDS: chronic inflammation of the lungs, bronchi, laser action, ultrastructure of the bronchial epithelium, autoradiography.

The beneficial effect of helium-neon lasers on patients has been used on an increasingly wide scale in recent years in medicine for the treatment of many diseases [3, 5, 6, 10, 13-15]. However, the mechanism of laser biostimulation remains largely unexplained [11], and for that reason this method is used purely empirically in clinical practice. The clinical importance of laser therapy necessitates an analysis of the experimental and clinical data in order to elucidate changes taking place in the tissues and cells of man and animals under the influence of laser radiation.

The aim of this investigation was to study structural and metabolic changes in the bronchial wall of patients with chronic inflammatory diseases of the lungs, during treatment with a helium-neon laser.

## EXPERIMENTAL METHOD

Altogether 98 biopsy specimens from the lobar and segmental bronchi of 39 men (from 2 to 6 bronchial biopsies were performed on each patient) aged from 28 to 62 years with chronic suppurative lung diseases were studied. In 27 cases (74 bronchial biopsies), besides the usual course of treatment, bronchoscopy was carried out with scattered irradiation of draining bronchi with an LG-75 helium-neon laser, emitting light continuously with a wavelength $\lambda$ = 632.8 nm; the output was 3 mW. The number of sessions depended on their therapeutic effect and varied from 2 to 6, with exposure of 3-5 min. Bronchial biopsy was performed before each session of laser therapy. The control group consisted of 12 patients with chronic inflammatory lung diseases treated by the traditional anti-inflammatory measures without laser therapy. Bronchial biopsy was performed on these patients before and after the course of treatment (24 biopsy specimens). The method of obtaining the biopsy material during bronchoscopy was described previously [7]. Most of the biopsy specimens were fixed in 10% neutral formalin solution. A tissue fragment measuring 1 $mm^3$ was incubated in medium 199 containing $^3$H-thymidine or $^3$H-uridine, and this was followed by autoradiographic analysis. Autoradiographs of semithin sections (exposure 5 days) were prepared by the method in [9, 12]. The part of the biopsy specimen which was not incubated was fixed in a 4% solution of paraformaldehyde and a 1% solution of $OsO_4$, and embedded in a mixture of Epon and Araldite. In each case paraffin, semithin, and ultrathin sections were cut. The paraffin sections were stained with hematoxylin and eosin together with Perls' reaction, by Van Gieson's method with staining of elastic fibers with Weigert's resorcinol-fuchsine, and the PAS reaction. Semithin sections were stained with azure II and Schiff's reagent, while the ultrathin sections were stained with uranyl acetate and lead citrate.

## EXPERIMENTAL RESULTS

Considerable pathological changes in the bronchial mucosa were found in bronchial biopsy specimens taken before the beginning of laser therapy in most patients. In a minority of

Laboratory of Ultrastructural Bases of Pathology, Department of Pathomorphology and Morphometry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk. Laboratory of Histochemistry and Autoradiography, Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 12, pp. 743-749, December, 1987. Original article submitted April 3, 1987.

0007-4888/87/0012-1755$12.50  

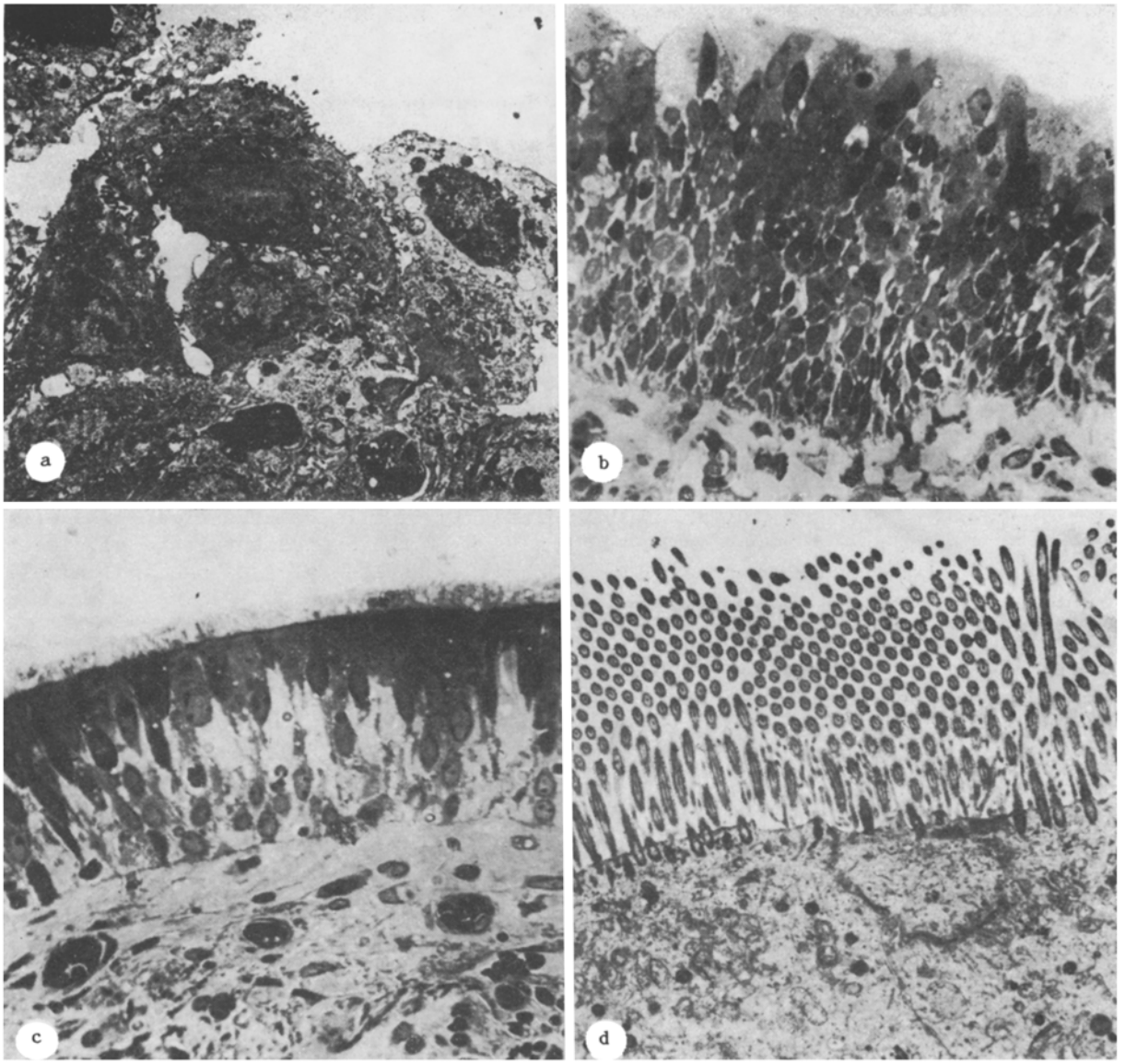


Fig. 1. Tissue and ultrastructural changes in bronchial epithelium in patients with chronic inflammation of the lungs treated by laser irradiation. a) Metaplasia of bronchial epithelium, absence of ciliary apparatus. 2000 ×; b) proliferation of basal cells and increase in height of epithelial layer. Semithin section, azure II. 400 ×; c) stratified cylindrical ciliated epithelium. Semithin section, azure II. 480 ×; d) regular arrangement of cilia on apical surface of epitheliocytes. 2600 ×. a) Biopsy before laser treatment, b-d) after helium-neon laser treatment.

cases, the bronchial epithelium preserved its stratified structure and the cylindrical shape of the cells, but various lesions of the ciliary apparatus were always observed. The most typical of them was a change in the configuration of the plasma membrane of the cilia, differences in the quantity of their matrix, and disorganization of their basal bodies and axial microtubules. The total number of cilia was greatly reduced and the regularity of their structure was disturbed.

Clarification of the cytoplasm, vacuolation of the cytoplasmic reticulum, and partial destruction of mitochondria were observed in the ciliated epitheliocytes. The degenerative changes affecting the ciliated cells were often considerable: the cytoplasmic matrix became translucent or electron-dense, the organelles in it were hard to distinguish and the nuclei underwent lysis or pycnosis. The number of goblet cells decreased and their secretory function was depressed. The basal cells often underwent hyperplasia. The intercellular spaces were widened.

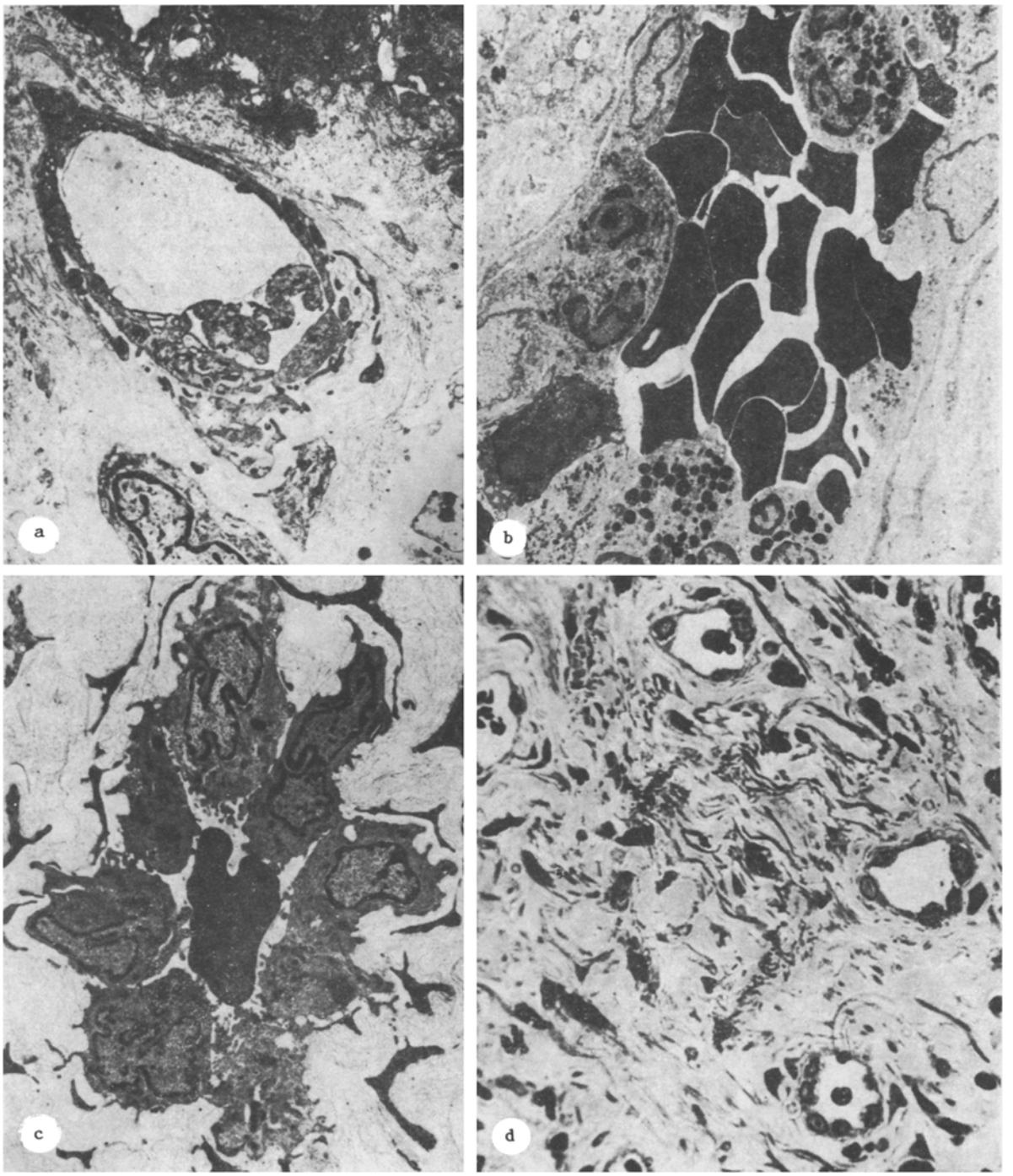


Fig. 2. Tissue and ultrastructural changes in lamina propria of bronchial mucosa of patient with chronic inflammation of the lungs treated by laser. a) Thinning of capillary wall, pericapillary sclerosis. 5000 ×; b) dilatation of capillary lumen, hyperemia, pavementing and diapedesis of leukocytes. 2600 ×; c) hypertrophy of endotheliocytes of a postcapillary venule, deep invaginations of nuclear membranes. 3000 ×; d) newly formed connective tissue with marked vascularization. Semithin section, azure II. 400 ×. a) Biopsy before laser treatment, b-d) after helium-neon laser treatment.

In most cases the bronchial epithelium was not differentiated into ciliated and goblet cells (Fig. 1a); the epithelial layer consisted of basal and intermediate cells, which underwent dystrophy on the apical surface or were transformed into typical squamous epithelial cells, i.e., they underwent epidermoid metaplasia [8]. At the ultrastructural level, sometimes isolated ciliated epitheliocytes were observed among the metaplastic cells.

The most typical changes in the lamina propria of the bronchial mucosa in all observations were sclerosis and a polymorphonuclear cellular inflammatory infiltration combined with acute disturbances of the hemodynamics.

On the whole the morphological picture of the mucosa in the bronchial biopsy specimens corresponded to that of chronic bronchitis with reduction of the cilia and metaplasia of the cylindrical epithelium stratified squamous.

In bronchi exposed to the action of the laser, considerable changes were discovered after 5-8 days in both the epithelium and the underlying stroma. Marked hyperplasia of the basal cells was most characteristic of the epithelium. The hyperplasia was so marked that the proliferating basal cells not only increased the height of the epithelial layer (Fig. 1b), but also formed polyplike outgrowths, which penetrated deeply into the underlying tissue.

The lamina propria of the bronchial mucosa at these times was strongly hyperemic. Many capillaries, whose dilated lumen was packed with erythrocytes and leukocytes (Fig. 2a, b), were observed. Marked diapedesis of the leukocytes was present with migration of neutrophils and lymphocytes from the lumen of the vessels into the perivascular tissue, and then diffusely throughout the mucosa. Many neutrophils and lymphocytes were found within the epithelium. The neutrophils stained intensely with Schiff's reagent. Ultrastructural investigation revealed many granules of two types in them: in some cells large electron-dense granules, related to lysosomal structures, predominated, but neutrophils more often contained specific granules.

The ultrastructure of the blood capillaries after laser irradiation showed some distinguishing features: the endotheliocytes were varied in structure, although on the whole they had ultrastructural features of actively synthesizing cells. Some cells around the perimeter of a capillary had long, tapering cytoplasmic processes, whereas other endotheliocytes were almost cubical in shape and formed short cytoplasmic outgrowths (Fig. 2c).

Nuclei of all the cells were enlarged and contained predominantly euchromatin and one to three large nucleoli with granular-fibrillary structure. Hyperplasia of the lamellar complex of the endotheliocytes, accumulation of filamentous structures in their perinuclear zone, accompanied by a marked decrease in size of the pinocytotic vesicles and absence of outgrowths on the basal and lamellar surfaces were noted.

The changes described above were often accompanied later by proliferation of blood capillaries lying parallel to each other, but perpendicularly to the basal membrane. Cellular infiltration in these regions was most polymorphic: besides neutrophils and lymphocytes, there were also macrophages, plasma cells, eosinophils, mast cells, and fibroblasts, i.e., granulation tissue with its cell composition dominated by neutrophils was formed.

The autoradiographic investigation at these times revealed increased metabolic activity of the epitheliocytes (Fig. 3a, c), endotheliocytes, and stromal cells. For instance, the labeling index with $^3$H-uridine was 52% for epitheliocytes before laser treatment, and increased to 86.9% after helium-neon laser treatment. In all cases virtually 100% of endothelial cells incorporated $^3$H-uridine. The proliferative response of the epithelium (as reflected in $^3$H-thymidine uptake) varied. Whereas in the original biopsy material the bronchial epithelium had a stratified squamous structure, 8-10 days after laser treatment the labeling index fell from 15 to 4.7%, but in other cases it doubled during this same period (Fig. 3b, d).

Changes in the epithelium in the submucosa continued for a varied length of time, but on the whole hyperemia and diapedesis of leukocytes were reduced 1 month after treatment, and this was accompanied by a corresponding decrease in the degree of cellular infiltration of the lamina propria. The qualitative composition of the infiltrating cells changed: many fibroblasts, with ultrastructural signs of cells actively synthesizing protein, appeared. Later collagen fibers forming thin, delicate bundles, appeared and gradually replaced the foci of granulations and of inflammatory infiltration (Fig. 2d). It must be emphasized that, by contrast with the initial state, the newly formed connective tissue had a higher content of ground substance and of thin, regularly oriented bundles of collagen fibers.

Synchronously with subsiding of the inflammatory reaction, structural changes took place in the stroma of the bronchial epithelium. Epithelial cells in the course of regeneration, as they passed through the stage of marked hyperplasia, began to appear like transitional epithelium, they then developed signs of pseudometaplasia and, finally, normal differentiation of the epithelium took place into stratified ciliated epithelium, in which the

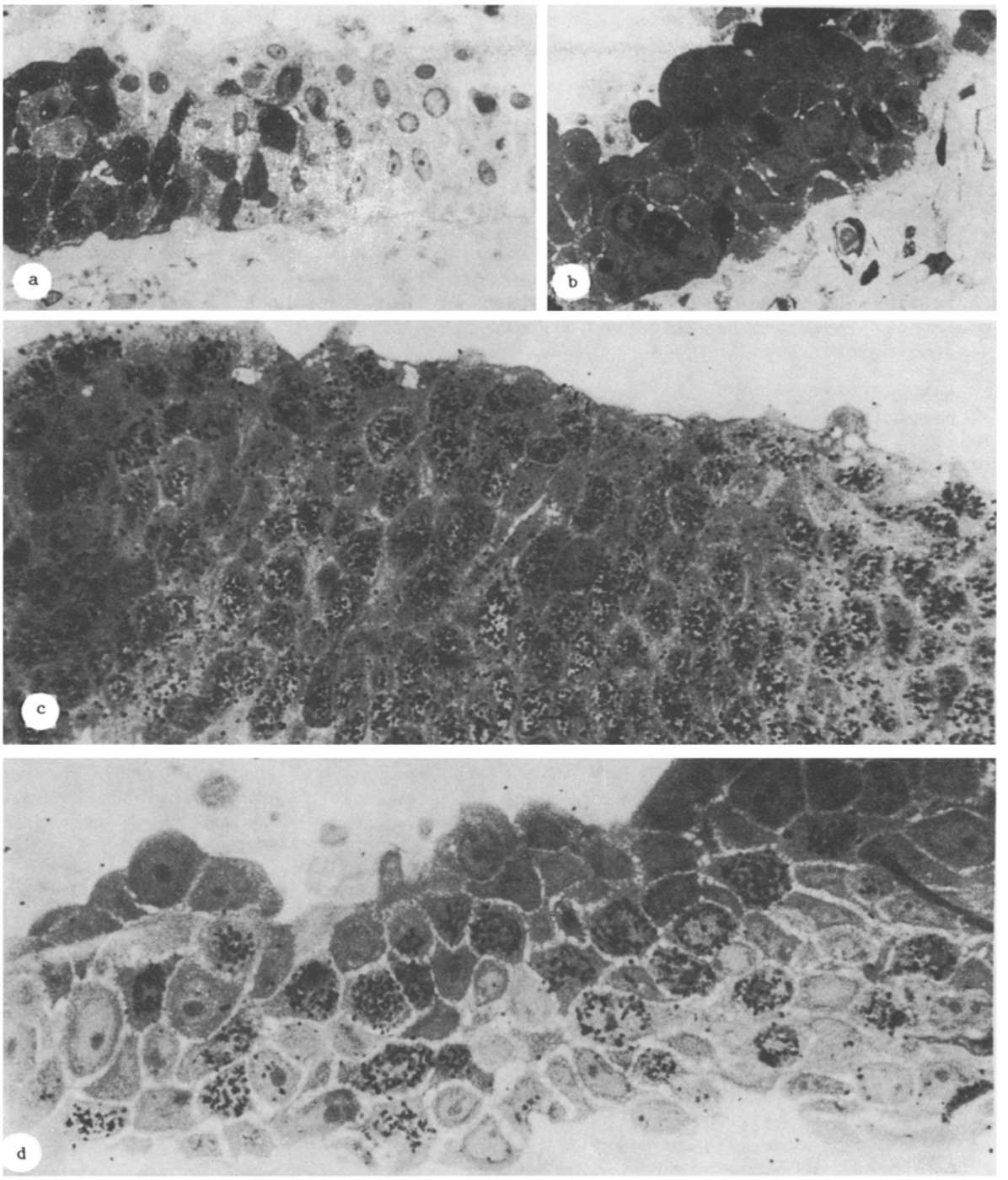


Fig. 3. Autoradiographic investigation of RNA and DNA synthesis in epithelium of large bronchi of patient with chronic inflammation of the lungs treated by laser. a) Single epitheliocytes labeled with $^{3}$H-uridine; b) single epitheliocytes labeled with $^{3}$H-thymidine; c) active RNA synthesis in bronchial epithelium; d) intensive DNA synthesis in bronchial epithelium. a, b) Biopsy before laser treatment, c, d) after helium-neon laser treatment. a, c) Incubation of biopsy material with $^{3}$H-uridine; b, d) with $^{3}$H-thymidine. a-d) Semithin sections, azure II. 400 ×.

ultrastructure of the goblet and ciliated cells was close to normal (Fig. 1c, d).

The autoradiographic study showed that the proliferative activity of the epithelial cells rose to a maximum, after which it showed a tendency to fall, and 30 days after laser treatment the labeling index of the epithelium with $^{3}$H-thymidine was 4.5%, compared with 22.3% in the endothelium; this parameter still remained high, as before, for RNA synthesis (98%).

The general pattern of structural changes in the bronchial wall during laser therapy, although generally stereotyped, varied in intensity and with the passage of time, depending on the character of the pathological process in the lungs, the intensity of the inflammatory reactions in the bronchi, the patient's age, and the number of sessions of laser therapy. The normal epithelial cover was sometimes restored as early as after 18-20 days, but in some cases this took up to 2 months.

The principal characteristics of the effect of laser therapy obtained in this investigation were therefore hyperplastic transformation with heterotopia of the bronchial epithelium and correlating changes in the structure of the underlying connective tissue. In their general interpretation, these observations are in agreement with the concept of parenchymal-stromal inter-relations, and of their particular variant, namely epithelial – connective tissue correlations [4]. This restructuring of the metaplastic bronchial epithelium, with modification of its differentiation and with restoration of the natural morphologic phenotype, while a pathological process still continues in the lungs, constitutes a unique phenomenon.

The investigation showed that there are certain procedures which can abruptly modify the program of structural organization of cells and tissues, and can thereby influence processes of proliferation [1, 2] and differentiation. The results of the study of structural-metabolic reactions in the bronchi during chronic inflammation, under the influence of laser stimulation, indicates that reorganization of the microcirculatory bed and, in particular, of the capillary, which is the natural pacemaker for the territory of the tissue microregion, are the primary changes. The intensity of response of the neutrophil population must also be noted in the morphogenesis of this process; these cells, infiltrating the mucosa and the intraepithelial tissue, are evidently important regulators of changes taking place in the bronchial wall.

## LITERATURE CITED


1. I. M. Baibekov and E. Musaev, Byull. Éksp. Biol. Med., No. 3, 95 (1983).
2. V. S. Barkovskii, Arkh. Patol., No. 8, 72 (1983).
3. N. F. Gamaleya, Lasers in Experimental and Clinical Medicine [in Russian], Moscow (1972).
4. V. G. Garshin, Inflammatory Hyperplasia of the Epithelium, Its Biological Importance and Relationship to the Problem of Cancer [in Russian], Moscow–Leningrad (1939).
5. I. N. Danilova, T. M. Kamenetskaya, A. A. Minenkova, and N. S. Airapetov, Vopr. Kurortol., No. 3, 36 (1978).
6. S. D. Pletnev (ed.), Lasers in Clinical Medicine [in Russian], Moscow (1981).
7. G. I. Nepomnyashchikh, Intravital Morphology of the Human Large Bronchi in the Presence of Chronic Inflammatory Diseases of the Lungs [in Russian], Novosibirsk (1977).
8. G. I. Nepomnyashchikh, Pathological Anatomy and Ultrastructure of the Bronchi in Chronic Inflammation of the Lungs [in Russian], Novosibirsk (1979).
9. G. I. Nepomnyashchikh, V. N. Efremov, L. M. Nepomnyashchikh, and V. P. Tumanov, Byull. Éksp. Biol. Med., No. 12, 744 (1985).
10. V. V. Polosukhin and A. N. Laivin, Adaptation of Man to Climatogeographic Conditions and Primary Prevention [in Russian], Vol. 1, Novosibirsk (1986), pp. 154-155.
11. A. A. Primak, M. V. Shesterina, N. I. Minitser, et al., Probl. Tuberk., No. 1, 57 (1986).
12. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Electron-Microscopic Autoradiography of the Cell [in Russian], Moscow (1980).
13. K. Häussinger and R. M. Huber, Internist (Berlin), 20, 221 (1985).
14. G. Lonauer and B. Krohn-Grimberghe, Med. Welt (Stuttgart), 36, 962 (1985).
15. E. Mester, L. Hazay, M. Fenyo, et al., Optoelektronik in der Medizin, Berlin (1982), pp. 146-152.