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ORIGINAL ARTICLE

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Dynamics of the ultrastructural changes in blood and lymphatic capillaries of bronchi in inflammation and following endobronchial laser therapy

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Abstract An ultrastructural and autoradiographic analysis of changes in 188 biopsy specimens of bronchial mucosa of the large bronchi from 76 patients with chronic inflammatory lung diseses was carried out. Fibrosis results in an apparent reduction of metabolic activity in endothelial cells, affecting the proliferation of basal cells with changes in cell differentiation. Endobronchial laser therapy with an helium-neon laser induces proliferative and metabolic processes in the lamina propria of the bronchial mucosa with hyperaemia, intensive diapedesis of leucocytes and formation of leucocytic infiltrations and granulation tissue. The proliferative and metabolic activity of endothelial and stromal cells increases, and delicate fibrous connective tissue is formed.

Key words Inflammation of the bronchi · Bronchial biopsy · Ultrastructure · Vessels · Laser therapy

Introduction

Low-energy laser irradiation is widely used in modern medicine [1, 3, 8], including the treatment of respiratory disease [5], and has recently been used in combination with bronchoscopy [8]. The thereapeutic effect of laser treatment appears to be associated with the induction of regeneration [5, 8]. However, the processes involved have not been studied in detail, and the mechanism of the biostimulatory influence is not clear [8].

These mechanisms have been investigated in the large bronchi by morphological analysis. Patients being treated for chronic inflammatory lung diseases of different types were studied, and particular attention was given to the investigation of ultrastructural changes in the lymphatic and blood capillaries.

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Materials and methods

A total of 188 biopsy specimens from the lobar and segmental bronchi of 76 patients with chronic inflammatory lung diseases (chronic destructive diseases and tuberculosis of lung) were studied. In 63 cases (162 biopsy specimens from bronchi) bronchoscopy was carried out with scattered irradiation of the bronchi using an LG-75 helium-neon laser continuously emitting light with a wavelength (L) of 632.8 nm; the output was 3 mW. The number of sessions depended on the therapeutic effect and varied from two to six, with an exposure of 3–5 min. The laser dose was determined by experimental results [9] and it has been shown that this pattern is optimal for mild biostimulation. Even a slight increase in dose results in damage to the components of the bronchial wall. Bronchial biopsy was performed before each session of laser therapy. The control group consisted of 13 patients with similar lung diseases, who were treated by traditional anti-inflammatory methods without laser therapy. Bronchial biopsy was performed on these patients before and after the course of treatment.

Most of each biopsy specimen was fixed in a 10% solution of neutral formalin. The part of the biopsy specimen intended for electron microscopy was fixed in a 4% solution of paraformaldehyde and a 1% solution of OsO_4 and embedded in a mixture of Epon and Araldite.

Autoradiographic study of bronchial biopsies by incubation of specimens with precursors of synthesis of DNA (^{3}H-thymidine) or RNA (^{3}H-uridine) was carried out [10]. Fragments measuring 1 mm^3 were taken from newly obtained biopsy specimens and placed in bottles with 5 ml of medium 199 and one of the radioactive precursors. The content of ^{3}H-thymidine (specific radioactivity 24 Ci/mM) was 100 mcCi/ml, and the content of ^{3}H-uridine (specific radioactive 26.6 Ci/mM) was 200 mcCi/ml. Fragments were incubated for 1.5 h at 37° C and then fixed in a 4% solution of paraformaldehyde and processed for embedding in Epon and Araldite. The number of tissue specimens for each group of patients is given in Table 1.

For the light-optical autoradiographic analysis, semithin sections were prepared and covered with photoemulsion of “M”-type (dilution 1:5). Slides were dried and exposed in the dark for 5 days at 4° C. The duration of developmen was 2 min and duration of fixing, 5 min. Preparations were stained with Azure 11. On semithin sections of bronchial biopsies, the percentage of labelled epithelial and endothelial cells was calculated as a labelling index (not less than 1000 cells were examined in each case).

In each case, paraffin, semithin and ultrathin sections had been cut. The paraffin sections were stained with haematoxylin and eosin together with Perls’ reaction, by Van Gieson’s method with staining of elastic fibres with Weigert’s resorcinol-fuchsin, and the PAS reaction. Semithin sections were stained with Azure 11 and Schiff’s reagent, while the ultrathin sections were stained with uran acetate and lead citrate.

Table 1 Autoradiographic analysis of RNA and DNA synthesis in bronchial epithelium and capillary endothelial cells of bronchial mucosa (numerator is the labelling index with ^{3}H-uridine; denominator is the labelling index with ^{3}H-thymidine)

Types of changes of epithelium	Labelling index (%)		
	Before treatment	6–8 days after	>20 days after
Bronchial epithelial cells			
I (*n*=3)	$\frac{60.3 \pm 4.8}{1.38 \pm 0.08}$		
II–III (*n*=8)	$\frac{68.3 \pm 4.2}{2.34 \pm 0.59^a}$	$\frac{73.2 \pm 5.2}{3.84 \pm 0.48^b}$	
IV Metaplasia (*n*=5)	$\frac{38.5 \pm 4.0^a}{8.41 \pm 0.87^a}$	$\frac{72.9 \pm 5.6^b}{3.98 \pm 0.24^b}$	$\frac{90.8 \pm 4.4^b}{2.78 \pm 0.73}$
IV Atrophy (*n*=4)	$\frac{33.9 \pm 6.6^a}{0.92 \pm 0.08^a}$	$\frac{77.3 \pm 6.0^b}{3.58 \pm 0.32^b}$	
Endothelial cells of blood vessels			
I (*n*=3)	$\frac{61.3 \pm 3.7}{-}$		
II–III (*n*=8)	$\frac{78.6 \pm 2.5^a}{-}$	$\frac{98.5 \pm 0.4^b}{-}$	
IV Metaplasia (*n*=5)	$\frac{46.9 \pm 4.4^a}{-}$	$\frac{98.3 \pm 0.3^b}{-}$	$\frac{99.8 \pm 0.1^b}{-}$
IV Atrophy (*n*=4)	$\frac{36.9 \pm 2.6^a}{-}$	$\frac{97.9 \pm 0.4^b}{-}$	

[a] Significant differences between labelling indices of the first form and subsequent forms of changes in bronchial epithelium (Student *t*-test; level of significance set at 5%)
[b] Significant differences between labelling indices before and after laser treatment (Student *t*-test; level of significance set at 5%)

Results

The classification of ultrastructural changes of epithelium of the large bronchi on chronic inflammatory lung diseases devised by Nepomnyashchikh [6] was used as the basis of the estimation of the extent of proliferative activity. All biopsy specimens were divided into four groups, representing sequential stages in the development of inflammatory process in the bronchial wall.

In the first group, vessels with signs of physiological circulation and normal functional activity (according to the level of RNA synthesis) were predominant in the capillaries. Lymphatic capillaries had wider lumina than those of the blood capillaries, and were of a complex irregular structure in cross-section because of numerous bends and folds of their walls.

Bronchial epithelium in these specimens was stratified, with normal ciliary apparatus. Incorporation of the label was observed in 1.38±0.08% of the cells of the bronchial epithelium after incubation of biopsy specimens with ^{3}H-thymidine. Basal-type epithelial cells were in the majority, and isolated goblet and ciliated cells were seen. Incubation of biopsy specimens with ^{3}H-uridine showed incorporation of the label into the epithelial, endothelial and stromal cells of connective tissue (Table 1).

In the biopsy specimens of the second and the third groups increased filling of the blood capillaries and local inflammatory infiltration consisting of lymphocytes and plasmocytes were seen in the lamina propria of the bronchial mucosa. Endothelial cells showed signs of hypertrophy and contained numerous micropinocytic vesicles in the cytoplasm. They formed many cytoplasmic processes on the basal and luminal surfaces (Fig. 1a). These cells actively incorporated the label during incubation with ^{3}H-uridine (Table 1). However, perivascular fibrosis was seen around some capillaries (Fig. 1b).

Lymphostasis and dilation of lymphatic lumina, correlating with the level of hyperaemia and oedema in the bronchial mucosa, were typical for the majority of lymphatic capillaries (Fig. 1c). The basal surface of the endothelial cells of such capillaries often formed numerous cytoplasmic outgrowths, and these cells had thinned lateral processes.

The second type reflected changes in ciliated epithelial cells and hyperplasia of goblet glandular cells. Alteration of the epithelial cells was predominant in the bronchial epithelium in the third type of ultrastructural lesions (Fig. 1d). Hypersecretion by goblet glandular cells was replaced by degeneration with decreased secretory function.

Increased activity in blood capillaries and intensification of endothelial cell division were accompanied by higher proliferative and metabolic capacity of the bronchial epithelial cells than in the first group (Table 1). These results led us to merge the autoradiographic data from the second and third groups of bronchial biopsy specimens.

Biopsy specimens with changes of differentiation in the bronchial epithelium were assigned to the fourth group. Epidermoid metaplasia was observed most often (Fig. 2a). In some cases the epithelium underwent atrophy with the formation of an epithelial layer of one or two layers.

Atrophy or fibrosis in combination with polymorphonuclear inflammatory infiltration on a background of microcirculatory bed reduction were the most typical changes in the lamina propria of the bronchial mucosa in this

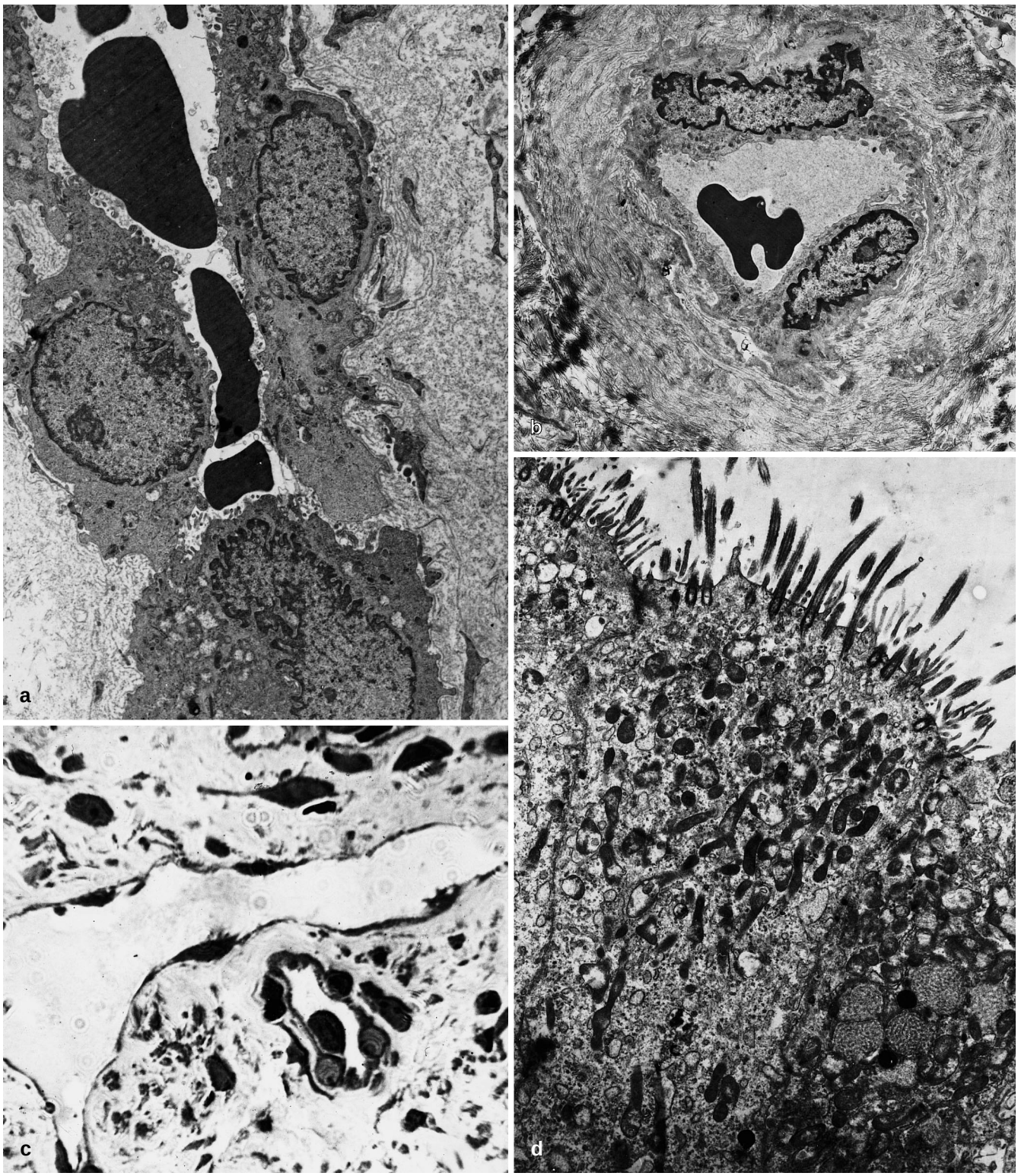
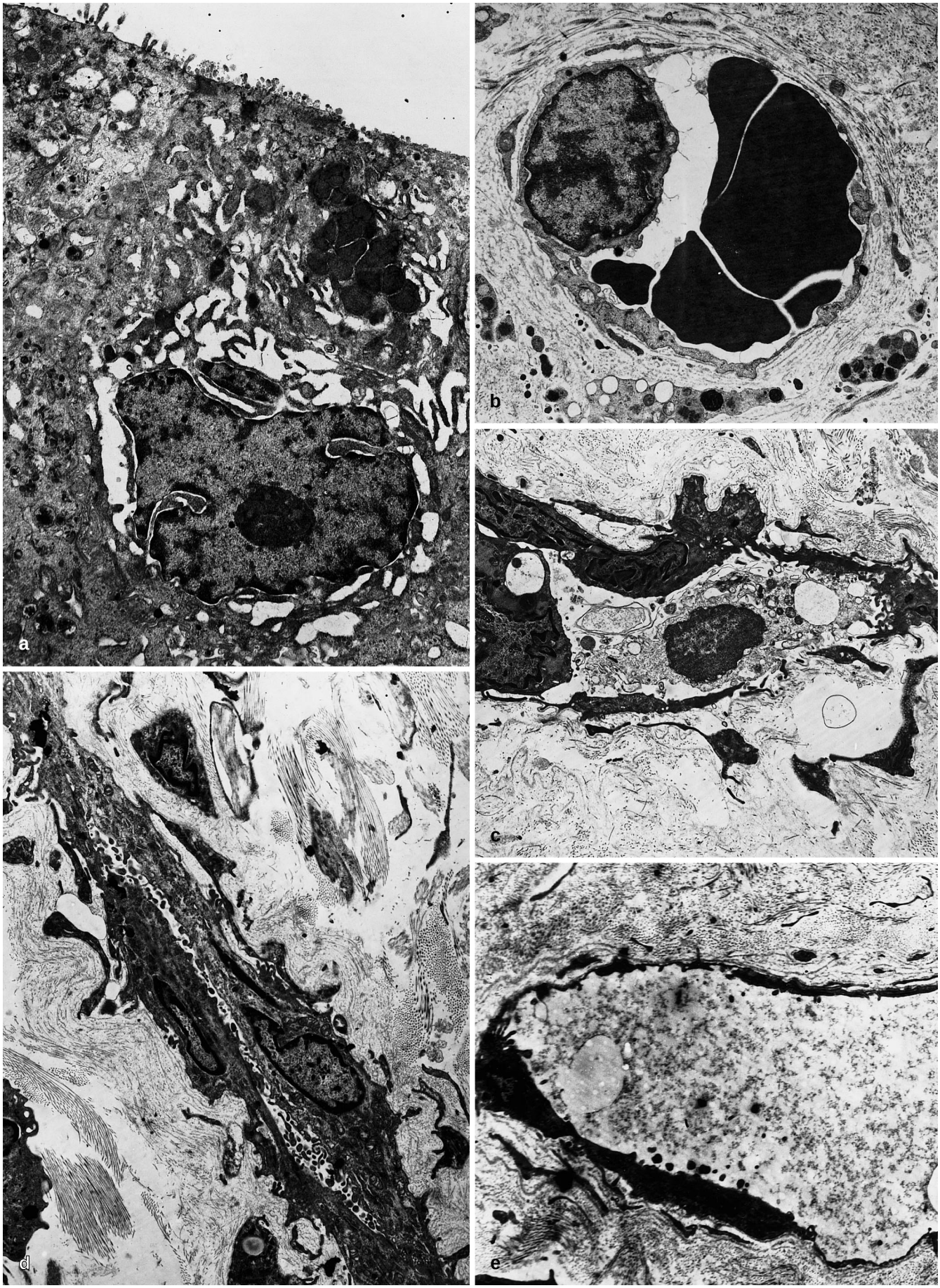


Fig. 1a Hypertrophy of capillary endothelial cells. ×3300. **b** Pericapillary fibrosis. ×2000. **c** Dilated lymphatic capillary. Semithin section, Azure II, ×800. **d** Alteration in ciliated epithelial cells. ×3300

group. The perivascular zone and basal layer underlying the bronchial epithelium showed extensive fibrosis. The endothelial cells of the majority of blood capillaries became thinner, and nuclei of endothelial cells protruded into the lumina of vessels (Fig. 2b). Protein aggregates, fragmented platelets and erythrocytes were found in some capillary lumina. These findings reflected the processes of local thrombosis of the microcirculatory bed. Some-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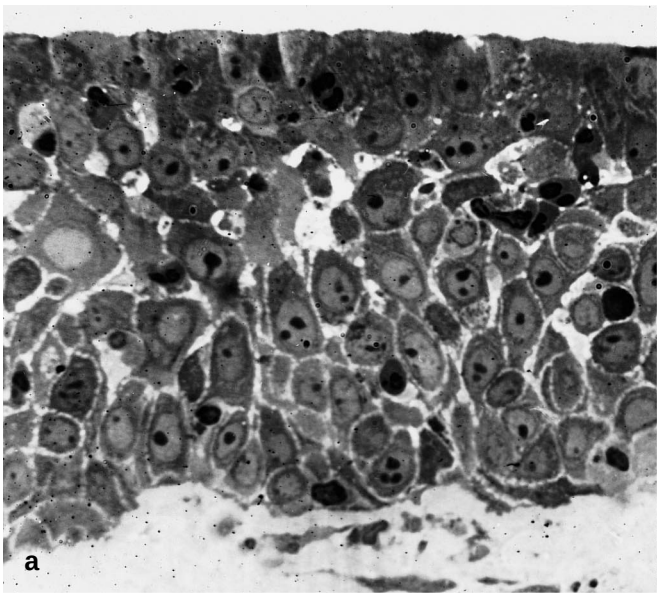

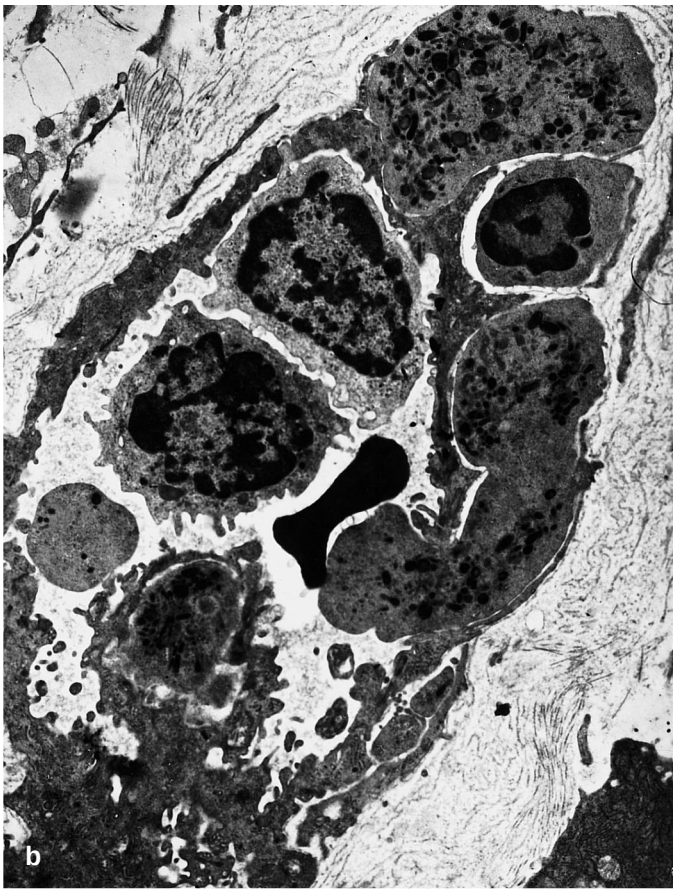

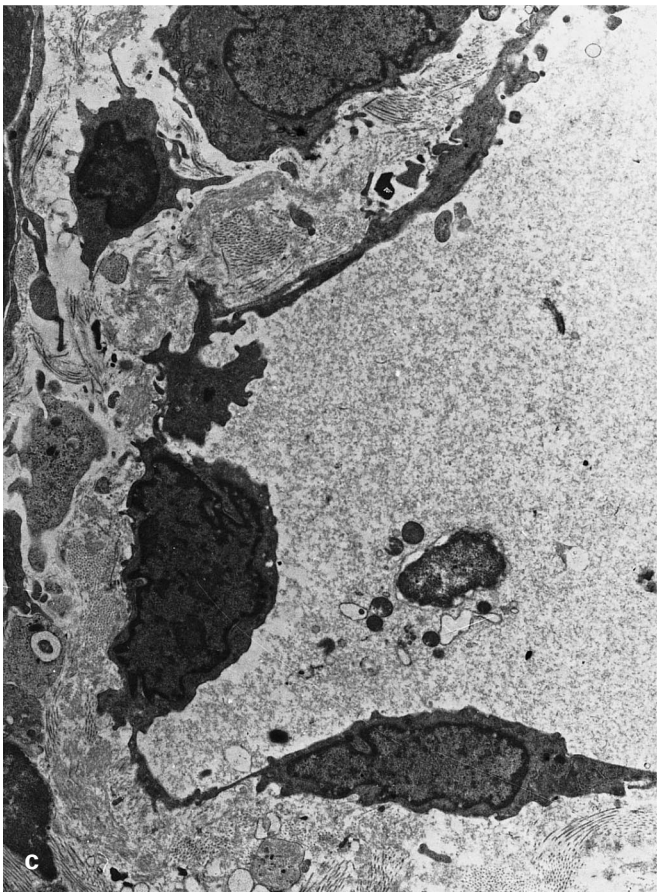

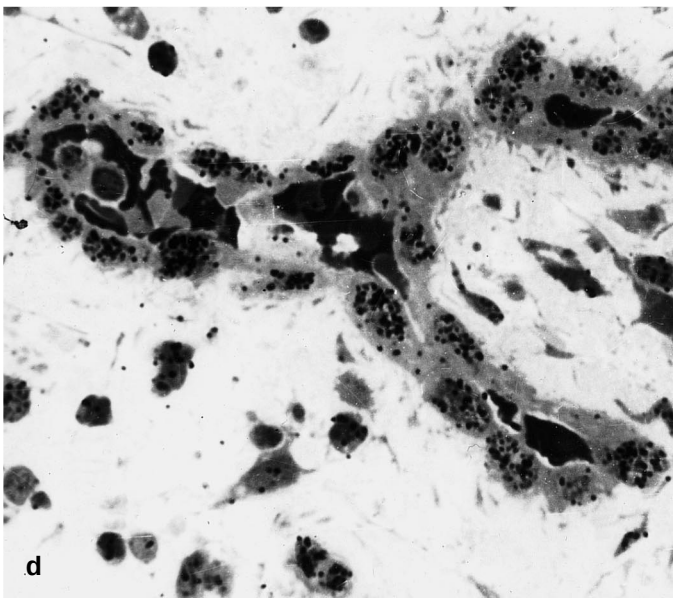

Fig. 3a Proliferation of bronchial epithelium. ×600. **b** Transendothelial migration of leucocytes. ×3300. **c** Lymphatic capillary. ×2000. **d** RNA synthesis in endothelial cells of the capillaries. ×1000. **a**, **d** Semithin section, Azure 11; **d** incubation of biopsy material with ^{3}H-uridine

◀ **Fig. 2a** Epidermoid metaplasia: surface epithelial cells. ×3300. **b** Thinning of capillary endothelial cells. ×3300. **c** Disruption of capillary endothelium. ×3300. **d** Collapsed lumen of postcapillary venule. ×2000. **e** Thinning of endothelial cells of a lymphatic capillary. ×2000

times capillaries with significant lesions in the endothelial cells and interrupted endothelium were found (Fig. 2c).

The majority of postcapillary venules were strongly contracted, but ultrastructural appearances in endothelial cells suggested heightened functional activity, these included invaginations of nuclear membrane, abundant intracellular organelles and many cytoplasmic processes (Fig. 2d).

Lymphatic dilatation reflected the intensification of congestion. The overwhelming majority of capillaries were distended, forming thin-walled cavities (Fig. 2e).

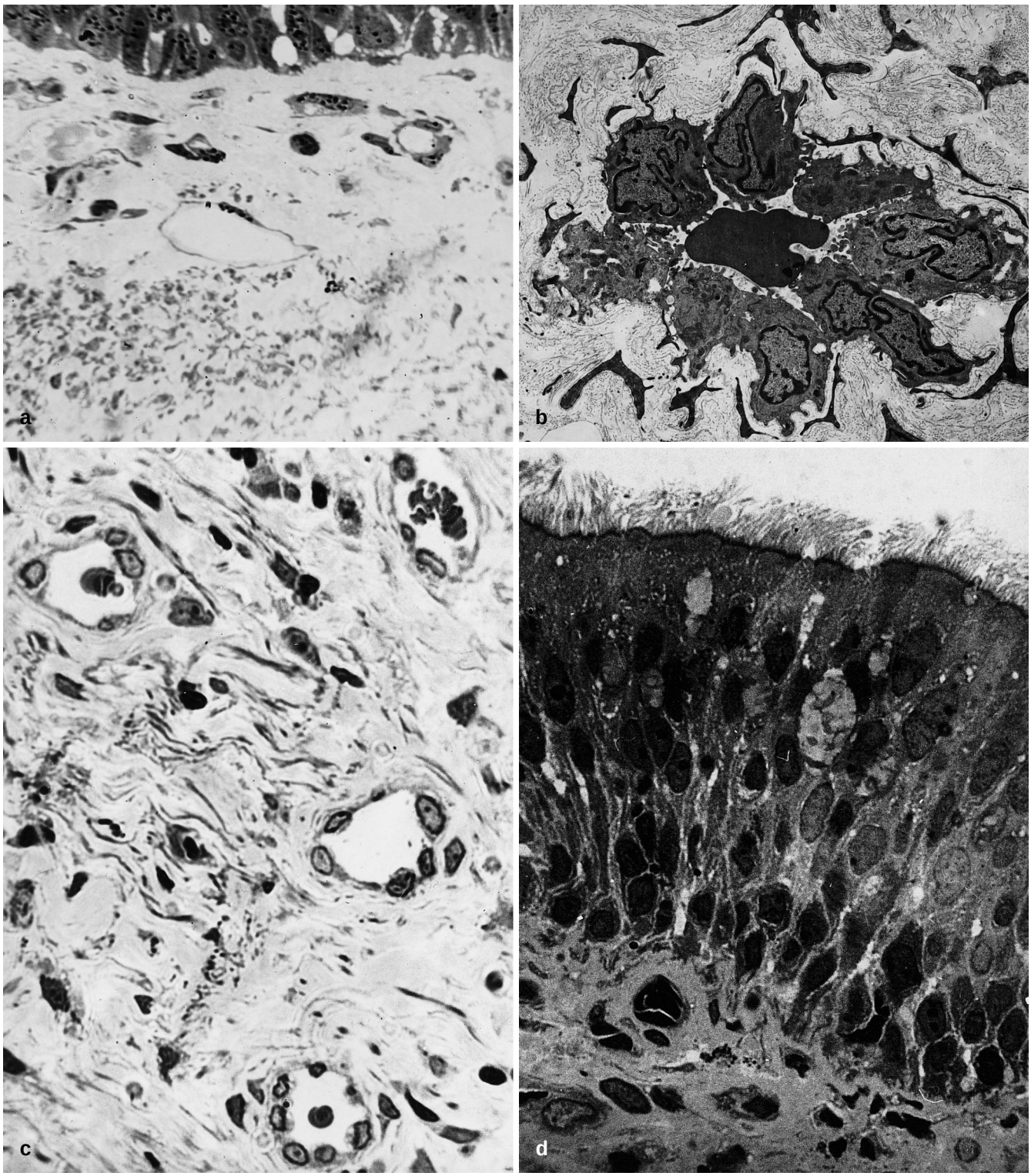

Fig. 4a RNA synthesis in the endothelial cells of a lymphatic capillary. ×800. **b** Postcapillary venule with high cubical endothelium. ×3300. **c** Newly formed connective tissue with marked vascularization. ×400. **d** Restoration of normal structure of epithelial cells of the bronchial epithelium. ×3300. **a**, **c**, **d** Semithin section, Azure 11; **a** incubation of biopsy material with ^{3}H-uridine

Autoradiographic analysis (Table 1) showed that structural changes were accompanied by decrease in RNA synthesis. Two changes in the proliferative activity of bronchial epithelium were found. The first showed a decrease of proliferative activity leading to atrophy. The second is characterized by increased proliferative activity with epidermoid metaplasia [7].

Changes in the epithelium and underlying stroma were found in bronchi 6–8 days after laser irradiation. Marked hyperplasia of the basal cells was typical (Fig. 3a). This change was so marked that proliferating basal cells not only increased the height of the epithelial layer but also formed outgrowths, which penetrated deeply into the underlying tissue. Basal cells formed several strata. This metaplasia was described as a temporary transitional state (see [7]).

The lamina propria of the bronchial mucosa was strongly hyperaemic. Many dilated and congested capillaries, packed with erythrocytes and leucocytes, were observed. Moreover, marked diapedesis of leucocytes was found (Fig. 3b) with diffusion into the bronchial mucosa. A large number of neutrophils was found in the intraepithelial position. The ultrastructure of the blood capillaries reflected the heightened functional load of these cells. The extent of the granulation tissue formed depended on the severity of the sclerotic changes before laser treatment.

Restoration of normal structure and signs of activity were typical for the lymphatic capillaries. Thin parts of lymphatic endothelial cells formed numerous small cytoplasmic processes (Fig. 3c).

Autoradiographic study revealed the increased metabolic activity of the epithelial, endothelial and stromal cells as assessed by this methodology. In all cases virtually 100% of the endothelial cells of bood (Fig. 3d) and lymphatic (Fig. 4a) capillaries incorporated ^{3}H-uridine. The proliferative response of the epithelium varied (Table 1). During these times, single endothelial cells and pericytes also incorporated ^{3}H-thymidine.

One month after the first laser treatment, hyperaemia and diapedesis of leucocytes in the bronchi usually decreased and the intensity of cell infiltration was reduced. Vessels with a high cubical endothelium (Fig. 4b), resembling the postcapillary vanules of the lymph nodes, appeared in the rehabilitative morphogenesis. This was accompanied by a change in the cell infiltrate to a lympho-plasmocytic type.

At the same time, the qualitative composition of the inflammatory infiltrate changed; many fibroblasts with ultrastructural signs of active protein synthesis and high levels of RNA synthesis appeared. Later, collagen fibres forming thin, delicate bundles appeared and gradually replaced the granulation tissue and inflammatory infiltrate. These processes were completed by forming of delicate fibrous connective tissue (Fig. 4c).

In synchrony with the subsidence of the inflammatory reaction, structural changes took place in the stroma of the bronchial epithelium. Epithelial cells in the process of regeneration passed through a stage of marked hyperplasia, with subsequent normal differentiation into stratified ciliary epithelium in which the ultrastructure of the goblet and ciliated cells was close to normal (Fig. 4d).

The proliferative activity of the epithelial and endothelial cells 25–30 days after laser treatment was slightly reduced. Despite the high level of RNA synthesis (90.8±4.4%), the labelling index with ^{3}H-thymidine in epithelium was 2.78±0.73% at these times.

The general pattern of structural changes during laser therapy, although generally stereotyped, varied in intensity and with the passage of time, depending on the character of the pathologic process in the lung, the intensity of the inflammatory reactions in the bronchi and the number of sessions of laser therapy.

Changes indicating restoration of the ultrastructure were not recorded in patients receiving traditional treatment. The effects noted in their bronchi were confined to attenuation of the changes in bronchial epithelial cells, and reduction of the hyperaemia and oedema in bronchial mucosa.

Discussion

Acceleration of cell renewal and the intensification of metabolism by bronchial epithelial cells of the second and the third groups of biopsy specimens occurs under conditions of increased metabolic activity of endothelial cells of blood capillaries and cells in the perivascular zone. It is evident that prolonged “irritation” or the bronchial wall causes an increase in cells metabolism, which in combination with proliferative reactions compensates the deficiency of cells, taking place as a result of the injury to it and its death.

However, chronic irritation of the bronchial mucosa is accompanied by a progressive deficiency of lymphatic and microvascular systems. Hypoxia inducing atrophic and fibrotic processes can result from these lesions. Metabolic activity in endothelial and pericapillary cells decreases, and these tendencies influence the processes of proliferation of basal cells, changing, first of all, the environment of the basal cells of bronchial mucosa. This results in a change of differentiation in the bronchial epithelium.

The principal effect of laser therapy is hyperplastic transformation of bronchial epithelium correlated with the structural changes in the underlying connective tissue. This is followed by restoration of the normal phenotype. Such reconstruction of capillary bed and bronchial epithelium is unique. It is especially valuable in cases with extreme expression of the pathologic process in the bronchi in combination with atrophy and metaplasia of the bronchial epithelium.

The restructuring observed in the microcirculation of the lymphatic and blood systems is a reflection of its activation. In this process lymphatic capillaries restore structural organization with concomitant normalization of draining. The reaction spectrum of the blood capillaries is slightly broader. Rapid blood-filling with the migration of leucocytes into the stroma and the subsequent proliferation of capillaries with the formation of granulation tissue are the classic signs of exacerbation of a chronic inflammatory process [11]. Restoration of the normal morphology by the exacerbation of inflammation is also a unique phemonemon.

The transformation in bronchial epithelium and submucosa occurred together with intensified migration of

lymphoid elements into stroma and intraepithelial space. Infiltration of lymphocytes into the lamina propria of bronchial mucosa closely followed the appearance of capillaries with a high cubical endothelium, resembling the endothelial structure of postcapillary venules of lymph nodes [4]. Such vessels appear in zones of lymphoid infiltration and allow extensive transendothelial migration. Disappearance of the infiltration is accompanied by a reduction in the number of these vessels [2].

The morphological dynamics of bronchial biopsies from patients with inflammatory lung diseases treated by the different schemes was compared. Laser therapy produced much better resolution than was obtained in the control group and led to restoration of the normal structure.

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