ELECTRON-AUTORADIOGRAPHIC STUDY OF THE WALL OF THE LARGE BRONCHI IN CHRONIC INFLAMMATORY LUNG DISEASE

G. I. Nepomnyashchikh, V. N. Efremov,
L. M. Nepomnyashchikh, and V. P. Tumanov

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In chronic inflammatory lung diseases of varied genesis, a combination of stereotyped tissue, cellular, and subcellular reactions develops in the large bronchi [4]. A detailed tissue and ultrastructural analysis of biopsy material taken at bronchoscopy and during operations has enabled the time course of these changes to be studied and the states of the large bronchi to be assessed morphologically.

The aim of this investigation was to study chronic bronchitis from the standpoint of parenchymatous-stromal interrelations, using light and electron-microscopic autoradiography.

EXPERIMENTAL METHOD

Pieces of the walls of the lobar and segmental bronchi, obtained by incision during bronchoscopy on 19 patients with chronic inflammatory lung disease were studied. From the sample of tissue thus obtained fragments measuring 1 mm^3 were incubated at 37°C for 1.5 h in medium No. 199, containing one of the following isotopes: ^{3}H-thymidine (100 μCi/ml), ^{3}H-uridine (200 μCi/ml), and ^{3}H-proline (50 μCi/ml). At the end of incubation the material was washed to remove unincorporated precursor with cold medium No. 199, then washed 3 times with phosphate buffer (pH 7.4), fixed with 4% paraformaldehyde solution and 1% OsO_4 solution, and embedded in a mixture of Epon and Araldite. Part of the biopsy material was not incubated but fixed in 10% neutral formalin solution and embedded in paraffin wax. In each case paraffin, semithin, and ultrathin sections were cut. The paraffin sections were stained with hematoxylin and eosin in combination withth Perles' reaction, by Van Gieson's method with staining for elastic structures with Weigert's resorcin–fuchsine, and the PAS reaction. Semithin sections were stained with azure II and Schiff's reagent, and the ultrathin sections were stained with uranyl acetate and lead citrate. Autoradiographs of semithin (exposure 5 days) and ultrathin (exposure 30 days) sections were prepared from blocks embedded in epoxide resins by the method in [6].

The number of cells of the bronchial epithelium incorporating the label was counted in semithin sections and the ratio between the number of labeled cells of each type and the total number of epitheliocytes was determined. Labeled cells of all populations were counted in the mucosa and the relative number of labeled cells of each population was calculated. The density of the label was determined in nuclei of cells of each type. During incubation of the tissue with ^{3}H-proline the number of grains of silver in the nuclei and cytoplasm of the cells was counted. Since a semithin section of the incubated fragments of bronchial walls contained many labeled cells, the method of random sampling of the fields of vision was used in the quantitative analysis.

The results were analyzed depending on changes in the wall of the large bronchus [4]: chronic catarrhal bronchitis, sclerosing (fibrous) and catarrhal-sclerosing bronchitis (intermediate form between them).

Laboratory of Ultrastructural Principles of Pathology, Department of Pathomorphology and Morphometry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical 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. 100, No. 12, pp. 744-748, December, 1985. Original article submitted March 12, 1985.

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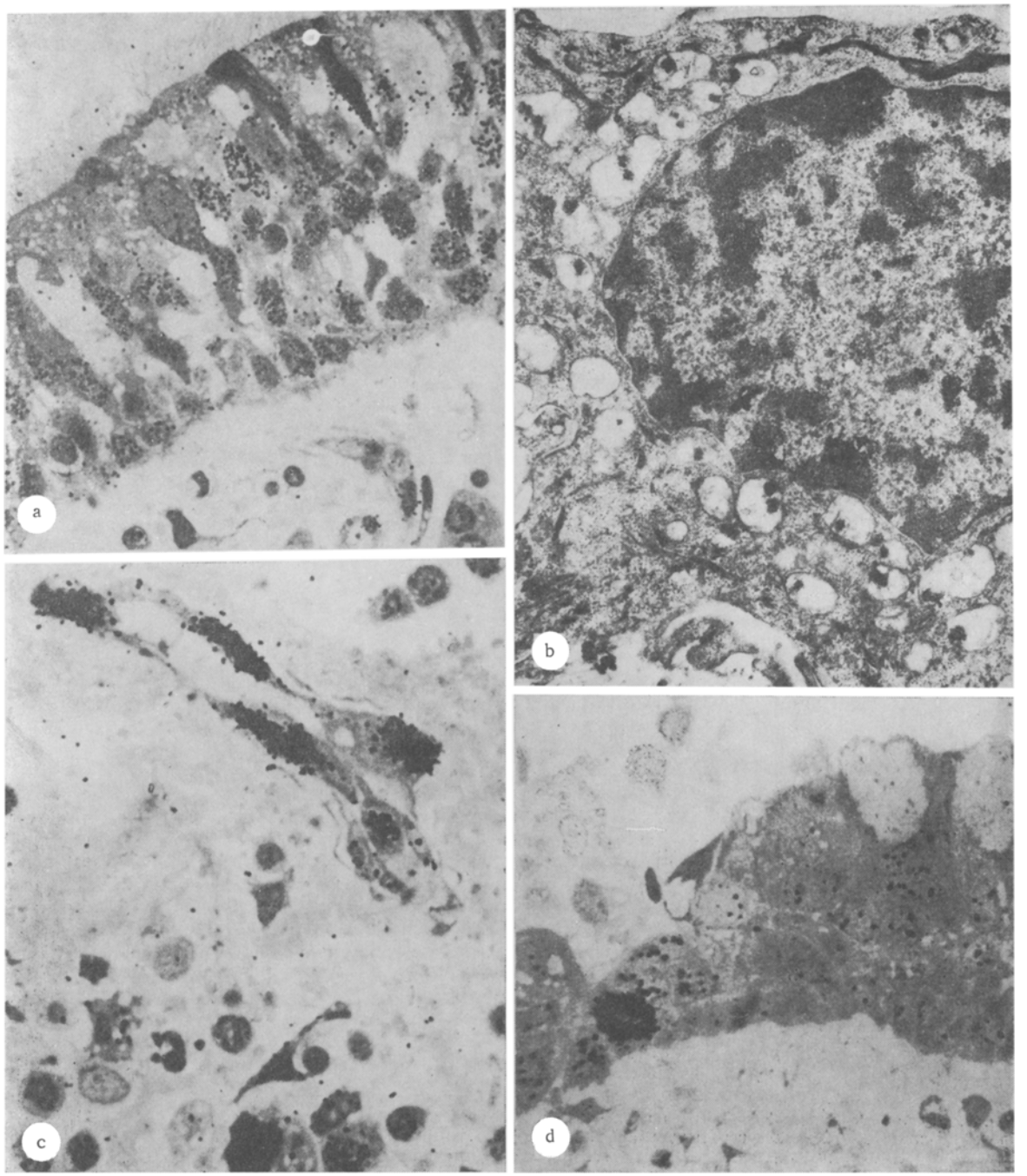

Fig. 1. Autoradiographic investigation of semithin and ultrathin sections from biopsy material taken from the large bronchi in chronic catarrhal bronchitis. a) High level of RNA synthesis in ciliated, goblet, and basal cells; b) moderate number of grains of silver above nucleus in basal cell of respiratory epithelium; c) high level of RNA synthesis in vascular endotheliocytes and relatively low level in cells of inflammatory tissue infiltrating lamina propria of mucosa; d) moderate DNA synthesis in basal and goblet cells of respiratory epithelium, absence or low level of DNA synthesis in epitheliocytes with disturbed ciliated cover. a, b, c) Incubation of tissue fragments with ^{3}H-uridine, d) with ^{3}H-thymidine. a, b, d) Semithin sections, stained with azure II, 100 ×, b) 15,000 ×

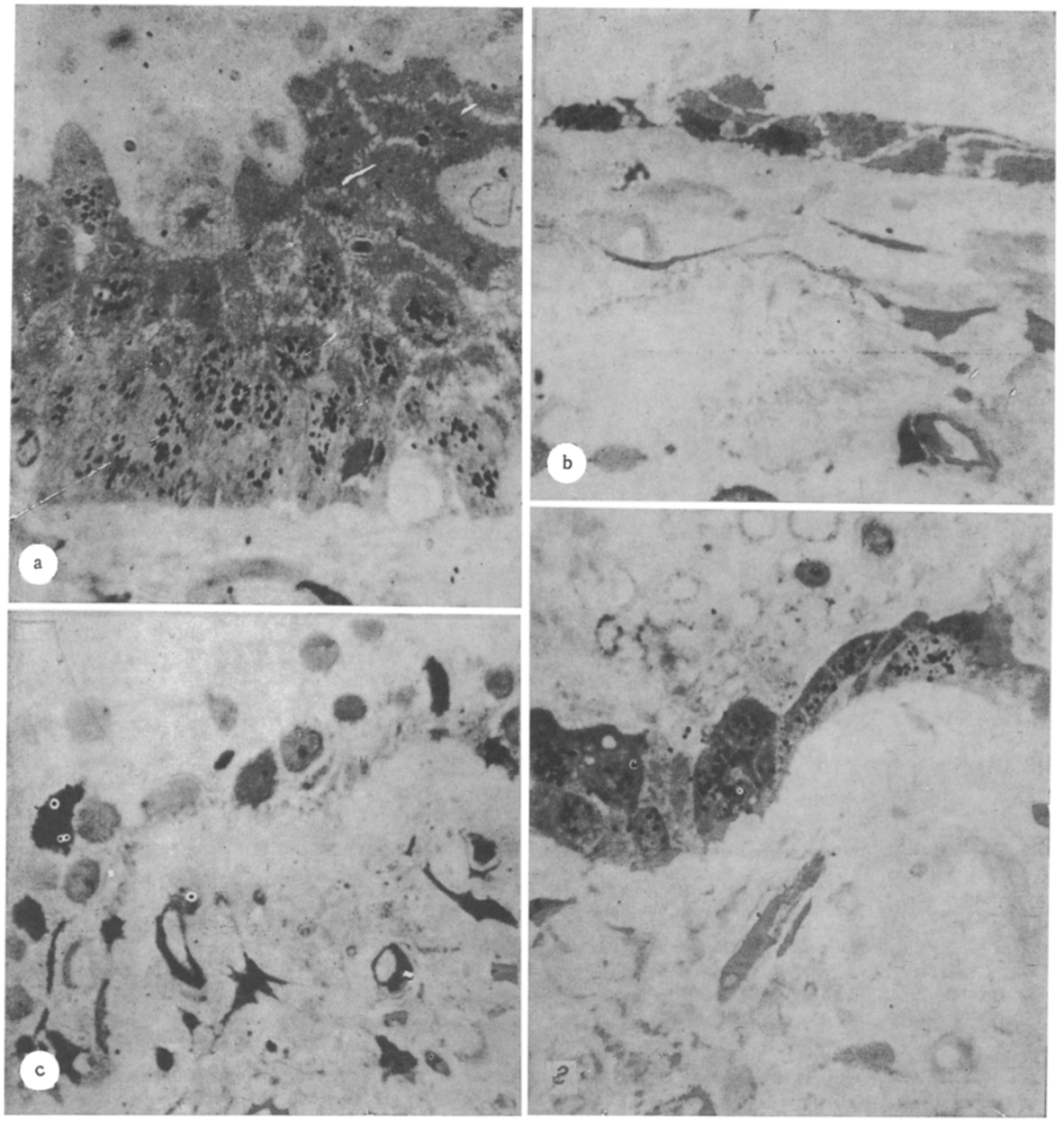

Fig. 2. Autoradiographic study of semithin sections from biopsy material from large bronchi in chronic sclerosing bronchitis (1000 x). a) Active RNA synthesis in branching basal cells and absence of RNA synthesis in dystrophically changed surface cells; b) atrophy of respiratory epithelium, RNA synthesis in flattened epitheliocytes of bronchial respiratory epithelium: single grains of silver above fibroblasts and above pericytes in lamina propria of mucosa; c) absence of DNA synthesis in endotheliocytes of vessels of lamina propria of mucosa and in cells of respiratory epithelium (grains of silver visible only above one epithelial cell); d) metaplasia of respiratory epithelium into classified squamous. Active DNA synthesis in basal epitheliocytes and capillary endothelium of lamina propria of mucosa. a, b) Fragments of tissue incubated with ^{3}H-uridine; c, d) with ^{3}H-thymidine.

EXPERIMENTAL RESULTS

Cell nuclei of the bronchial respiratory epithelium and also cell nuclei of the connective tissue and vascular endothelium of the lamina propia of the muscosa in biopsy material incubated with ^{3}H-uridine contained the label.

In chronic catarrhal inflammation the overwhelming majority of epitheliocytes were metabolically active: 100% of nuclei of ciliated and goblet cells and 87% of the basal cells contained label (Fig. 1a, b). The intensity of labeling was highest in the granulocytes (20.4 grains of silver), and rather lower in the ciliated (19.6) and basal (15.6) cells. The largest number of grains of silver was found above granulocytes, in which the lamellar complex was grossly hyperplastic, the rough endoplasmic reticulum well developed, and many granules of secretion liberated onto the surface of the epithelium were observed. Incidentally, many ciliated epitheliocytes with disturbance of the ciliated cover, with cytoplasmic outgrowths on the apical surface, a vacuolated endoplasmic reticulum, and focal destruction of mitochondria, were characterized by a high level of incorporation of ^{3}H-uridine. This is evidence of active metabolic processes taking place in cells exposed to an increased functional load, and aimed at restoring the lost structures. These functional characteristics of the cell and the high level of its metabolism indicate that these structural changes cannot be classed as dystrophic [1, 2].

During the transition from catarrhal to catarrhal-sclerosing information, i.e., during the development of sclerosis of the wall, the labeling index in ciliated and goblet cells fell (60 and 48% respectively) and the labeling density was reduced (8.2 and 6.7). The intensity of labeling in the nuclei of the basal cells showed a tendency to rise (Fig. 2a). During changes in the bronchial wall which may lead to a fibrous form of bronchitis, the secretory and ciliated epitheliocytes were solitary or absent, and the density of labeling with ^{3}H-uridine in the basal cells was low (down to four grains of silver).

Among cells of the lamina propria of the mucosa the endotheliocytes were labeled most intensively and constantly with ^{3}H-uridine. In chronic catarrhal inflammation the label was found in 100% of nuclei of these cells. A high labeling index was observed in macrophages (95%), mast cells (90%), and pericytes (80%). This index was much lower in fibroblasts (50%), plasma cells (17%), and lymphocytes (14%). The density of labeling was highest (up to 20 grains of silver) in endotheliocyte nuclei (Fig. 1c).

With an intensification of the sclerotic changes in the bronchial wall the index and density of labeling with ^{3}H-uridine fell in the endotheliocytes,pericytes, macrophages, and mast cells, but rose in the fibroblasts (from 50 to 82%) and plasma cells. Qualitative analysis of semithin sections showed that most labeled cells in the lamina propria were located in the pericapillary zone, and their number decreased away from the capillary.

On incubation of biopsy material with ^{3}H-thymidine incorporation of the label was found into basal, goblet, and solitary ciliated cells (Fig. 1d). The label was incorporated most actively in their lamina propria of the mucosa by endotheliocytes (40%) and fibroblasts (35%).

On the transition from chronic catarrhal to sclerosing bronchitis two tendencies were found in the behavior of the basal cells of the epithelium: in some cases a high index of labeling with ^{3}H-thymidine was preserved, whereas in others it fell sharply. Analysis of the material showed that these tendencies predetermine the subsequent structural changes in the bronchial respiratory epithelium: either metaplasia into stratified squamous epithelium or atrophy. In sclerosing bronchitis with atrophy the respiratory epithelium of the large bronchi consisted of one or two layers of flattened cells, 80-90% of which incorporated ^{3}H-uridine (Fig. 2b); however, DNA synthesis in the epithelium was drastically reduced (Fig. 2c). These structural changes were usually accompanied by low metabolic and proliferative activity of the endotheliocytes and stromal cells.

Sclerosing bronchitis with metaplasia of the epithelium into stratified squamous was characterized by a high labeling index on incubation of the tissue with ^{3}H-thymidine, most of which was incorporated into the basal cells of the epithelium and the endotheliocytes (Fig. d2).

After incubation of the tissue with ^{3}H-proline label was found in the endotheliocytes, pericytes, macrophages, and epitheliocytes, but the highest level of incorporation was observed in the fibroblasts in catarrhal-sclerosing and fibrous bronchitis. No grains of silver could be found above the polymorphonuclear leukocytes present in foci of chronic inflammatory

infiltration of the mucosa or migrating into the epithelial layer, on incubation with labeled uridine and proline, evidence of the low level of protein synthesis in these cells, and it correlates with the results of autoradiographic studies by other workers [8].

The results give a more complete idea of the morphogenesis of chronic sclerotic changes in the human bronchi. It is becoming clear that when the bronchial wall is subjected to prolonged "irritation" many of its cells switch to a higher level of metabolism, and in conjunction with proliferative reactions [5, 10, 11], this compensates for the deficiency of cells resulting from their death. However, after a certain time the structural-metabolic reserves of the cells become exhausted, their protein-synthesizing function is depressed, and changes in differetiation take place: the simple cylindrical ciliated epithelium is replaced by stratified squamous.

Heterotopic differentiation may arise, it can be tentatively suggested, in two ways: 1) basal cells are transformed and give rise to a new category of cells; 2) under the influence of an inducing stimulus new precursor cells are introduced into the system, they colonize it, and give rise to a new differetiation. These are good grounds for such an interpretation because any area of tissue of the internal medium in animals is to a high degree an open system. Evidence has now been obtained to show that repopulation processes are widespread in tissues of the internal medium [9].

From this standpoint the high metabolic and proliferative activity of the endotheliocytes and cells of the pericapillary zone assumes special significance. The dynamics of functional activity of the vascular cells is synchronized with metabolism and morphogenesis of the epithelial cells. This confirms the idea that the capillary plays a key role in relations between parenchyma and stroma on the territory of the tissue microregion [3]. It can also be postulated that during chronic irritation of the bronchial wall the vascular cells serve as the source for the formation of new stromal cells. Further evidence in support of this principle has been obtained by the study of some connective-tissue tumors [7].

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