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Atrophy of Bronchial Epithelium: Ultrastructural-Metabolic Analysis of the Bronchial Mucosa in Chronic Bronchitis

G. I. Nepomnyashchikh, L. A. Naumova, and L. M. Nepomnyashchikh

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Complex morphological analysis of bronchial biopsy specimens shows two forms of the atrophic process in the bronchial mucosa: the primary dystrophic form, whose basis is the syndrome of regenerative-plastic insufficiency, and the primary inflammatory form developing at the end of chronic inflammation.

Key Words: *bronchi; epithelial atrophy; chronic bronchitis; electron microscopy; autoradiography*

Atrophy has been little studied as a general pathological process. Atrophic states have been described predominantly on the basis of traditional macro- and microscopy, while descriptions of the ultrastructure of cells for different forms of atrophy are few [1,5,7]. Nevertheless, there is much about this phenomenon which remains debatable, calling for an in-depth analysis using modern methods, specifically autoradiography [7]. Bronchial epithelium responds stereotypically to various injuries. These changes are underpinned by a broad and complex spectrum of epitheliocyte phenotypic expression, including hyperplasia, stratification, metaplasia into stratified squamous epithelium, and anaplasia [2,10-15]. It has been found that, in addition to the above-mentioned structural and functional reorganizations of epithelium of the major bronchi, atrophy of the bronchial epithelium also underlies the morphogenesis of chronic pathological processes of the respiratory tract, notably chronic bronchitis [4]. Clinical endoscopy was previously used by us in studies of the atrophic forms of chronic bronchitis. The aim of the present study was to perform an ultrastructural analysis and autoradiography of the bronchial epithelium in atrophy of epitheliocytes.

Laboratory of the Ultrastructural Basis of Pathology, Research Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Ninety-two bronchial biopsies were performed in patients with a clinical diagnosis of "chronic bronchitis" during diagnostic bronchoscopy in cases where diffuse atrophy of the bronchial mucosa was detected by endoscopy. A BF B3 Olympus bronchofibroscope (Japan) was used.

For light-optic examination bioptates were fixed in 10% neutral Formalin. Paraffin sections were stained with hematoxylin-eosin and after Van Gieson with staining for elastic fibers using Weigert's resorcin-fuchsin, and the Schiff-iodine acid (SIA) reaction was performed.

For electron-microscopic investigation a fragment of bronchial bioptate was fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide, and after routine treatment embedded in Epon-Araldite. Semithin sections were stained with 1%

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azure II and by the SIA reaction, and examined under a light microscope. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined in a JEM 100B electron microscope.

Bronchial specimens to be used in autoradiography were incubated with the radioactive precursors of RNA and DNA synthesis (^{3}H-uridine and ^{3}H-thymidine, respectively) [6]. Fragments of bioptates were incubated in medium 199 with ^{3}H-uridine (26.6 Ci/mM, 200 μCi/ml) and ^{3}H-thymidine (24 Ci/mM, 100 μCi/ml) at 37°C for 1.5 h. After incubation specimens were routinely embedded in epoxy resins. The index of labeled nuclei was counted in semithin sections.

RESULTS

Light-optic examination of bronchial bioptates revealed atrophy of the bronchial epithelium attended by a marked reduction of the thickness of the epithelial layer. The latter comprised one or two layers of stratified or cubical cells partially preserving some ciliary bundles and small solitary granules of SIA-positive secretion. Atrophy of the integumentary bronchial epithelium in both groups was associated with foci of metaplasia and with the development of diffuse sclerosis in the lamina propria (Fig. 1, *a* and *b*).

Microscopic examination enabled us to distinguish between two groups of specimens of the bronchial mucosa. The absence of inflammatory cellular infiltration was typical of the first group, whereas chronic inflammatory infiltration of varying severity, in some cases with signs of transepithelial migration of leukocytes, was characteristic of the mucosa in the second group. It is noteworthy that focal hyperplasia of goblet and basal cells, more pronounced sclerotic changes of the vessels of the microcirculatory bed and lamina propria, and other morphological signs of chronic inflammation (polypoid growth of the bronchial mucosa and destroyed elastic structures) were observed in some bioptates of this group. Ultrastructural analysis revealed common tendencies in the pattern of changes in the bronchial bioptates of these two groups. These was a tendency of the ciliary apparatus and of the major cytoplasmic organelles to be reduced (Fig. 1, *c* and *d*) nuclear alterations, and increasing cell disintegration in the epithelial layer along with a decrease in the number of cell-to-cell contacts.

Regular distribution of cilia was disturbed in ciliary epitheliocytes; cilia were replaced with microvilli and cytoplasmic outgrowths. In the epinuclear zone of the cytoplasm the number of mitochondria markedly dropped; many of them had a cleared matrix and had partially lost their cristae; light, "balloon"-shaped mitochondria were observed. In the majority of cells the elements of the cytoplasmic reticulum were markedly fragmented and vacuolized; reduction of the granular cytoplasmic reticulum was noted; structures of the Golgi apparatus were rarely encountered.

Changes in goblet cells manifested themselves in transformation from a state with a lowered secretory activity to a state where the specific function was lost. The volume of goblet cells was reduced; a few elements of the lamellar apparatus and cytoplasmic reticulum were detected in the cytoplasm, and a small number of denser than normal granules of secretion surrounded by abundant lysosomes and autophagosomes were found in its epinuclear portion.

As dystrophic changes developed, cytoplasm disorganization progressed in the cells; the number of autophagosomes, which are heterogeneous structures surrounded by a membrane, increased.

Marked ultrastructural changes were noted in the nuclei. Nuclei with deep invaginations of the nuclear membrane predominated; in the epitheliocyte nucleoli, notably in specimens of the first group, segregation of the fibrillar and granular components was observed; ring-shaped and collapsed nucleoli were encountered.

Some structural peculiarities were noted in the vessels of the microcirculatory bed. In specimens of the first group vessels with a thinned endothelial lining predominated. Endotheliocytes had a dark, electron-dense cytoplasm. The nuclear heterochromatin content increased; the apical plasmalemma mainly had regular contours, solitary outgrowths being occasionally observed. Signs of pinocytosis were virtually absent. In bioptates of the second group the vascular endothelium looked swollen. The apical plasmalemma formed numerous microprocesses and invaginations. Abundant pinocytic microvesicles lay under the apical membrane.

The method of autoradiography has proved most important for assessing the structural-functional state of the bronchial mucosa in diverse forms of the pathological process [3,6].

The results of autoradiography of RNA synthesis in the structural elements of the bronchial mucosa in bioptates of the first group testified to a marked decrease in the protein-synthesizing function of bronchial epitheliocytes (Fig. 2, *a* and *b*) correlating with a lowered level of biosynthetic processes in the cell elements of the underlying stroma. The index of ^{3}H-uridine labeling in bronchial epithe-

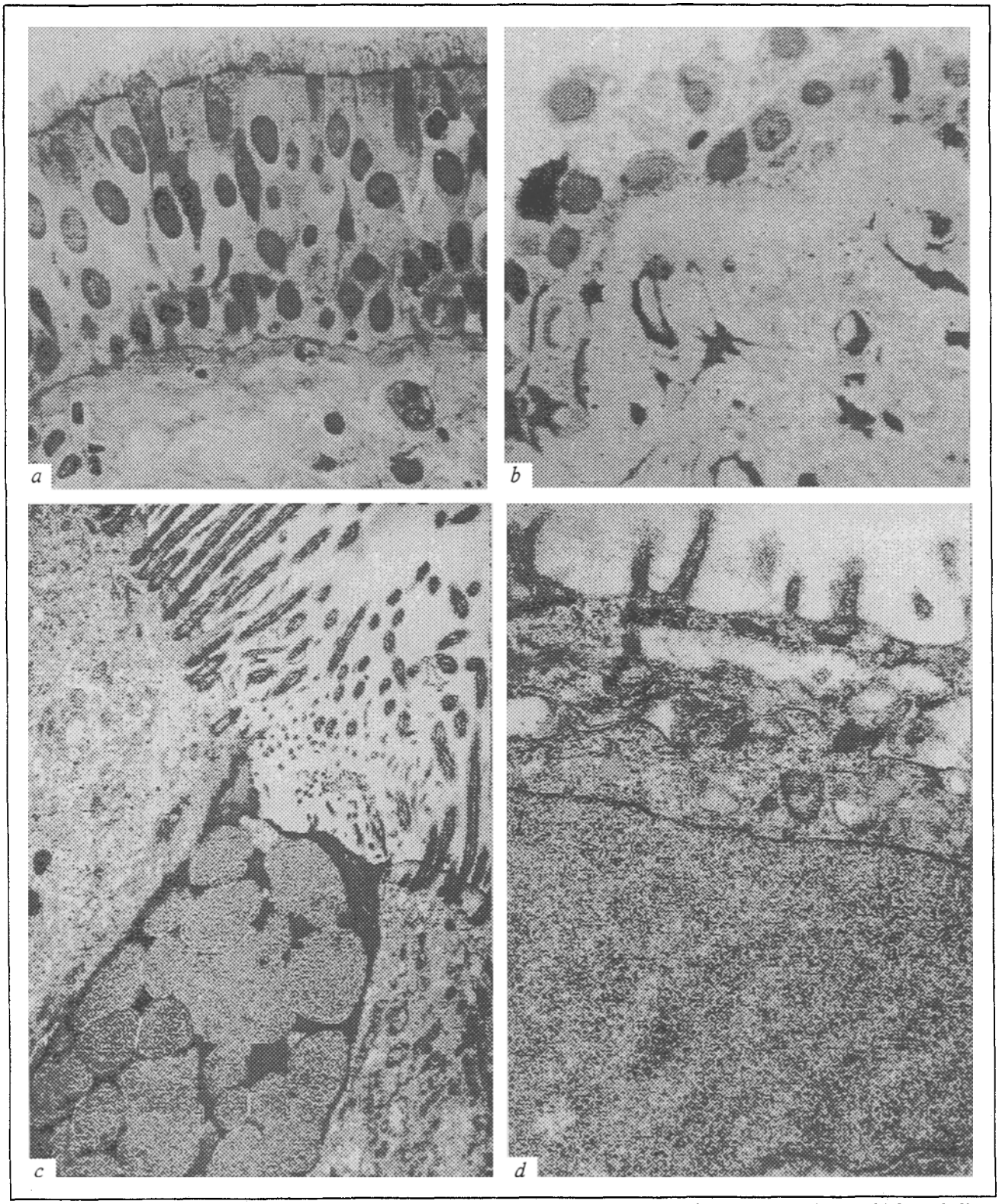

Fig. 1. Light-microscopic and ultrastructural characteristics of atrophied bronchial epithelium. *a*) bronchial epithelium of normal structure; semithin section; azure II staining; ×630. *b*) atrophy of bronchial epithelium; sclerosis of lamina propria in the absence of inflammatory-cellular infiltration; semithin section; azure II staining; ×1000. *c*) ciliary and goblet cells with normal ultrastructure; ×14,000. *d*) epitheliocyte atrophy: microvilli on the apical surface in the absence of cilia; ×10,000.

liocytes varied from 9.6 to 39.6% (mean value 26.8±12.2%). The index of labeled nuclei in epitheliocytes was from 54.3 to 81.7%, constituting 68.2±9.8% on average. Solitary granules of reduced sil-

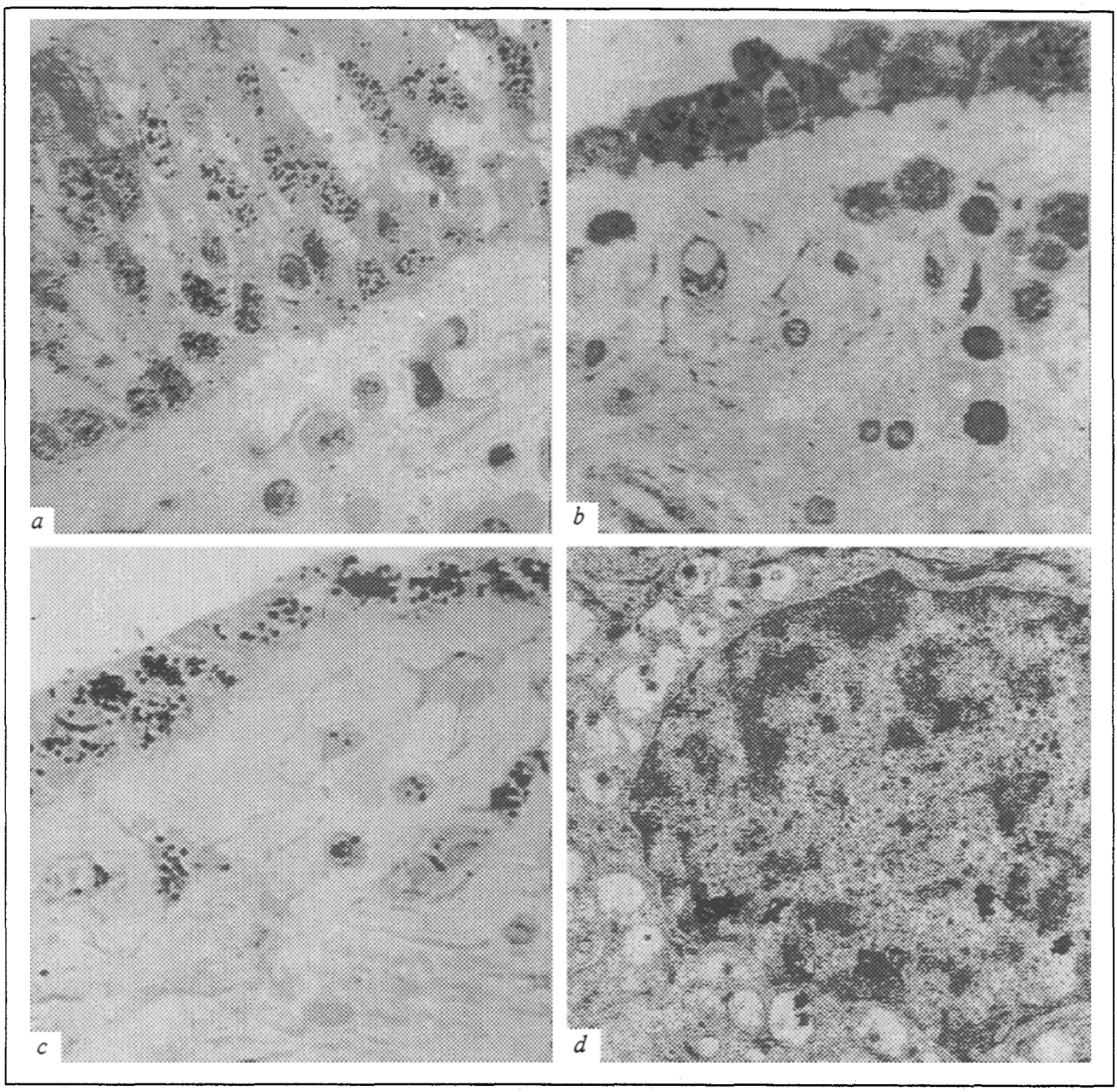

Fig. 2. Autoradiography of atrophied bronchial epithelium. Incubation with ^{3}H−uridine. *a*) high level of RNA synthesis in cells of stratified cylindrical ciliary epithelium; *b*) low level of RNA synthesis in atrophied bronchial epitheliocytes; *c*) high density of ^{3}H−uridine label in atrophied epitheliocytes; *d*) silver granules in nucleus of basal epitheliocyte. *a*−*c*) semithin sections, azure II staining, ×1000; *d*) ×6000.

ver were detected in the nuclei of some fibroblasts, lymphocytes, and macrophages of the subepithelial zone.

The metabolic activity of integumentary bronchial epithelium in bioptates of the second group was markedly higher (Fig. 2, *c* and *d*); the index of ^{3}H-uridine labeling varied from 36.3 to 87.5%, the mean value being 63.3±9.9% and the label density being rather high. In this group we also noted a positive correlation between the level of RNA synthesis in epitheliocytes and endotheliocytes. The index of ^{3}H-uridine labeling in the latter was from 64.3 to 82.4% (mean value 72.6±4.4%).

Autoradiographic analysis of the proliferative activity of the bronchial epithelium in specimens of the first group demonstrated that only a few cells incorporated ^{3}H-thymidine; in bioptates of the second group the index of ^{3}H-thymidine labeling markedly varied, being 1.5±0.8% on average.

Thus, a comparative analysis of structural changes in bronchial biopsy specimens provided evidence that atrophy of bronchial epithelium may not only occur at the end of chronic inflammation, but also develop as a transformation of primary and progressing dystrophy of the integumen-

tary bronchial epithelium into its atrophy with synchronous sclerosis of the bronchial wall and of the vessels of the microcirculatory bed. The described structural reorganization is attended by a marked reduction of biosynthesis in the cellular elements of the bronchial wall, which is documented by autoradiography. Such a pattern of structural-metabolic reaction attests to the development of the syndrome of regenerative-plastic insufficiency [4,8], which culminates in the decline of regenerative processes.

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