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Bronchial secretions and bronchial mucosa in children with cystic fibrosis: comparison of bronchoscopic, biochemical, bacteriological, microscopic and ultrastructural findings

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In children (mean age 12.1 ± 2.9 years) with cystic fibrosis, 44 bronchoscopic examinations were done under general anaesthesia with muscle relaxation using a Friedel type ventilation bronchoscope. The endoscopic picture of the mucous membranes was compared with the state of the bronchial secretions, its bacteriologic findings and content of acid mucopolysaccharides and DNA fibres (semiquantitative estimations). In all patients biopsy of the mucous membrane (central part of the bronchial tree) was performed for light and electron microscopy. The degree of reddening, swelling of the mucous membrane and hypersecretion was in some agreement with the intensity of the cellular infiltration and the production of pus (microscopic investigation). Secondary ultrastructural changes were detected in nearly all children, consisting of cellular oedema, swelling of mitochondria, dilatation of the endoplasmatic reticulum, protrusion of cells and fusion of cilia, enlarged intercellular spaces, thickening of the epithelial basal membrane, increased number of goblet cells, microtubular abnormities of the cilia, lesions of the apical cell membrane with loss of cilia and microvilli. These ultrastructural changes were not correlated with the above-mentioned signs of inflammation.

Except from deaths in the neonatal period due to meconium ileus, lung lesions are the major factors contributing to morbidity and mortality of cystic fibrosis (CF). The chronic pulmonary alterations in CF start from the trachea and the main bronchi [14]. Therefore, the aim of the present study was to obtain more information about the changes of the bronchial mucosa and their relation to the bronchoscopic picture and the characteristics of bronchial secretions. The study continues our earlier bronchologic studies in CF patients [7, 8, 9, 22].

MATERIAL AND METHODS

The subjects were 22 children, 14 boys and 8 girls from 7 to 17 years of age, with slight to moderate cystic fibrosis ascertained by repeated sweat tests, who were for years under our outpatient treatment. All these patients underwent bronchoscopic investigations under general anaesthesia with barbiturates and muscle relaxation with succinylcholine by Friedel's ventilation bronchoscope [10, 20]. The children's parents and in most cases the patients themselves gave informed consent to the examination.

The endoscopic picture (reddening and swelling of the bronchial mucosa, localized or generalized hypersecretion) was recorded. Samples of secretions were in-

vestigated by bacteriology and thin secretion films stained with acridine-orange dye for detection of DNA fibres (Bürgi method [3]) and with toluidine blue [21] to show the content of acid mucopolysaccharides (aMPS). In all patients biopsy of the mucous membrane was done at the central part of the bronchial tree with the aid of a special forceps. The specimens were kept in buffered 8% formalin for light microscopy or immersed in 3% glutaraldehyde-cacodylate buffer for 2 hours and post-fixed in 1% osmium tetroxide for 2 hours for electron microscopic investigation.

Results

From the clinical point of view, the severity of CF was estimated with Shwachman's score [14]: 10 patients had scores from 95 to 100, and the remaining 12 patients had values between 75 and 94.

The bronchoscopic picture showed in 35 of 44 examinations a slight (15), moderate (23) or severe (1) reddening of the bronchial mucosa, 34 times a swelling of the mucous membranes, in 34 of 44 examinations a slight (20) or heavy (14) localized hypersecretion and 16 times a generalized secretion. The secretions were in 20 cases purulent and in 14 cases seromucous.

These data were taken together into an own bronchoscopic score with a 13 stage scale; 4 times the finding was normal (score 1), 22 times slight inflammatory changes (score 2 to 5), 17 times moderate (score 6 to 9) and in 1 patient severe (score 11) alterations were observed (Fig 1).

The bacteriological examination showed 13 times sterile secretions, 26 times one strain of bacteria and 5 times a mixed bacterial infection. Staphylococci were found most often (25 times), other bacteria were Haemophilus influenzae, pneumococci, enterococci and Staphylococcus epidermidis (each of them found in two

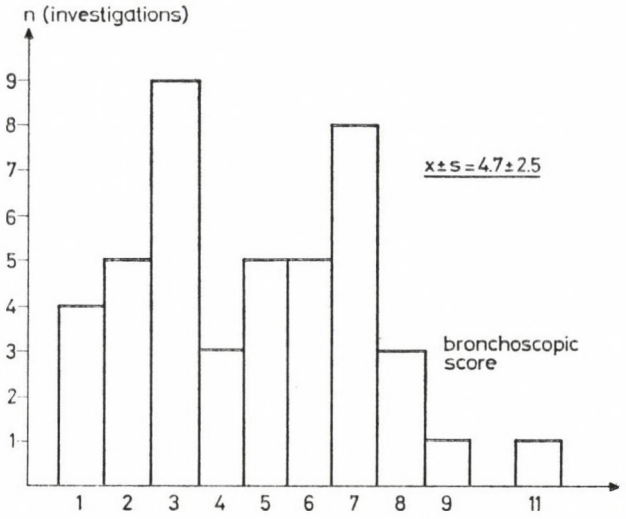


Fig. 1. Distribution of bronchoscopic score (degree of endobronchial inflammation) in 44 bronchoscopic examinations in children with cystic fibrosis. See text for details

cases) and Gram-positive cocci (one case). Pseudomonas aeruginosa was never detectable in these patients.

Semiquantitative evaluation of DNA and aMPS in the secretions was given in an 8 stage scale from 0 to +++ with intermediate stages.

From the findings the following correlations were calculated (r = coefficient of correlation):

clinical severity (Shwachman's score)/endobronchial inflammation (own bronchoscopic score) —r = 0.32,

DNA fibres in bronchial secretions/bronchoscopic score — r = 0.31, aMPS in bronchial secretions/bronchoscopy score — r = 0.34 and DNA fibres/aMPS in bronchial secretions — r = 0.59.

The highest (worst) mean bronchoscopic scores were recorded in children with staphylococci in the bronchial secretions (Fig 2), so the mean content of DNA fibres and aMPS was higher in these children than in those with sterile secretions or other bacteria found in it.

If the biopsy material was too small for both light and electron microscopy, we gave priority to the latter. In 21 of 44 examinations was the material sufficient for histological

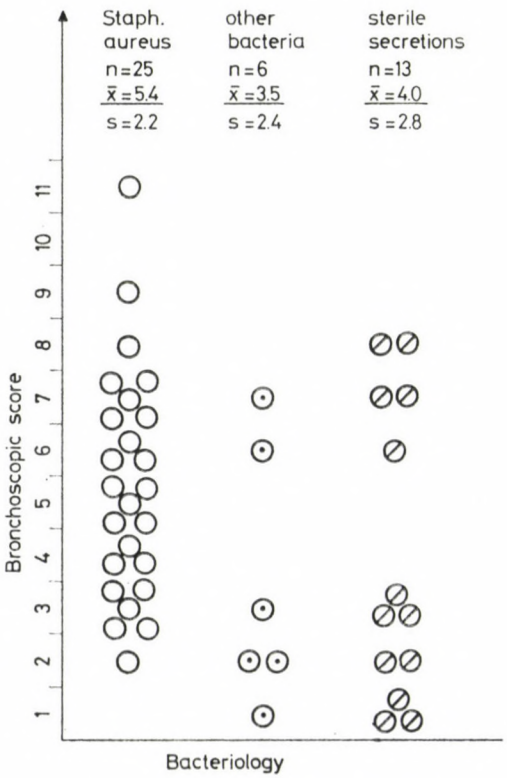
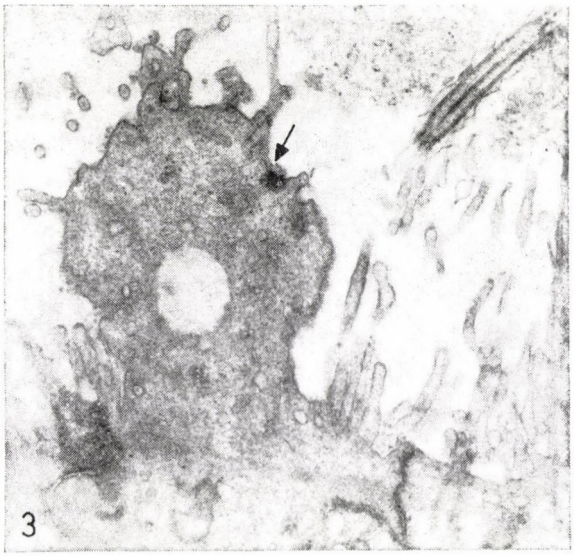


FIG. 2. Bacteriological findings of the bronchial secretions in 44 bronchoscopic examinations of children with cystic fibrosis, compared to the bronchoscopic score (Fig. 1)

examination and only in 16 cases could a clear decision be given about the degree of inflammation of the mucosa. We found twice a severe, 8 times a moderate and 3 times a slight chronic round cell infiltration and in 3 cases no inflammatory activity. With the exception of 3 children, the moderate or severe chronic inflammatory alterations were found in those



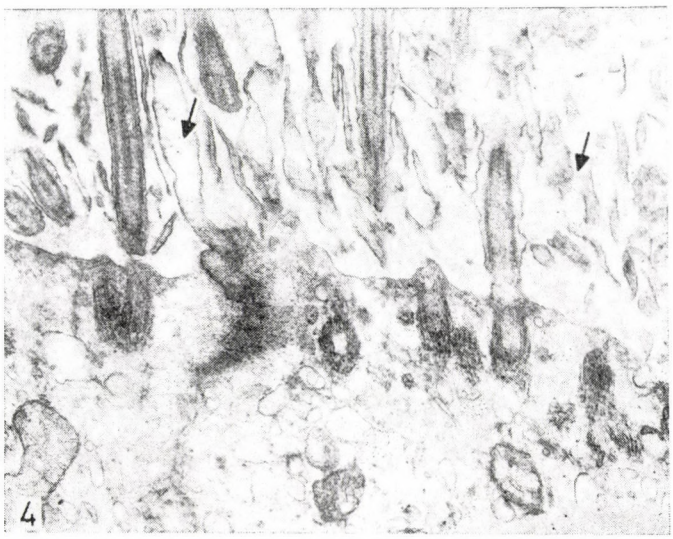


FIG. 3. Transmission electron micrograph of ciliated epithelium in a child with cystic fibrosis, showing protrusion (↓) of a cell with local destruction of cilia and microvilli. Magnification, 1:28 000

FIG. 4. Longitudinal-sectional profile of microvilli with degenerative changes (swelling and decay ↓). Magnification, 1:36 000

who showed a high degree of inflammation (i.e. high bronchoscopic score). Only in one case was the material sufficient for evaluation of the submucous glands. These showed signs of dyscrinia.

The material sufficed for ultrastructural investigation in 34 of 44 cases. Predominantly we evaluated the ciliated epithelium. The situation was as follows: no important changes (9 times), ciliary fusion with or without transposition of microtubuli (27 times), swelling and/or defect of the ciliary membrane (36 times) and cellular oedema (31 times). In more detail, the ciliary epithelium displayed

cellular oedema with swelling of mitochondria and dilatation of the endoplasmatic reticulum;

protrusion of cells (Fig 3) with alteration of cilia and microvilli (Fig 4);

enlargement of intercellular spaces;

penetrating inflammatory cells (lymphocytes and granulocytes) between the epithelia; and

thickening of the epithelial basal membrane.

As nonspecific lesions of the cilia we recorded:

alterations of their membrane (megacilia, swollen and fusioned cilia, protrusions, decay and complete loss of the ciliary membrane) (Figs 5, 6, 7);

microtubular abnormities (abnormal arrangement of aconemata after ciliary fusion (Figs 6, 7); and

faded granulary drawing of microtubuli.

There were in addition

an increased number of goblet cells (Fig 8) with granula full of mucus;

many dense granulary structures of mucus caused probably by its increased viscosity;

frequent cell fragments and inflammatory cells in the mucus;

lesions of the apical cell membrane with loss of cilia and/or microvilli, leading to a flat surface; and

alteration of microvilli (swelling and loss) and disturbance of the fluid transport.

None of these findings were correlated to the degree of inflammation (bronchoscopic score), the bacteriological or biochemical findings of the secretions or the inflammatory signs in light microscopy.

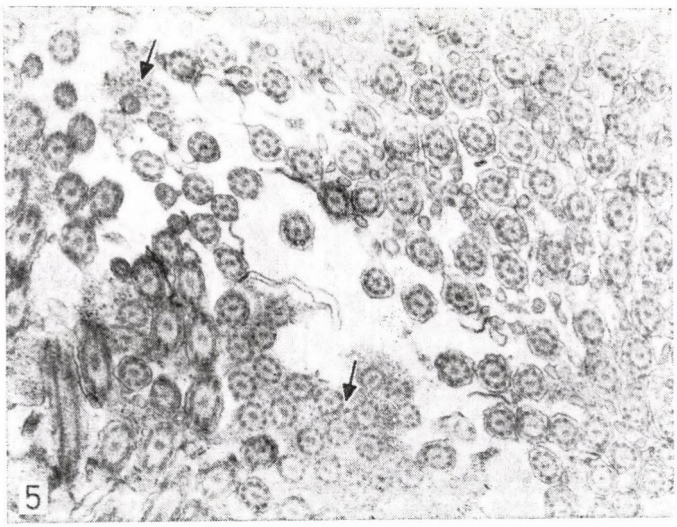
Discussion

It is well known that the severity and course of CF show a great variability among different patients. Although all the patients had a favourable course and only a mild form of CF (high Shwachman score and no Pseudomonas aeruginosa infection [14]), in most of them we could detect a pathologic endoscopic picture, pathologic findings of the bronchial secretions, chronic inflammatory changes in light microscopy and severe ultrastructural alterations.

There was only a slight correlation between the clinical severity and the bronchoscopic findings, between bronchoscopy and the content of DNA fibres and aMPS in the bronchial secretions, as well as between these semiquantitative biochemical parameters and the results of bacteriology.

Patients with staphylococcal infection had findings worse than those of the other children.

According to Chace et al [4] whose CF patients had a higher variability of the Shwachman score (from 5 to 80), there is a correlation between the content of hexose or sialic acid in bronchial expectorations and the severity of CF. It seemed that the light microscopic findings, i.e. signs of chronic infiltration with round cells, were in some agreement with the bronchoscopic picture of the mucosa. The alterations change intensely within weeks or months (see Fig. 1) due perhaps to intercurrent infections or exacerbations. Thus, bronchoscopic and histologic findings in CF are reflecting the degree of



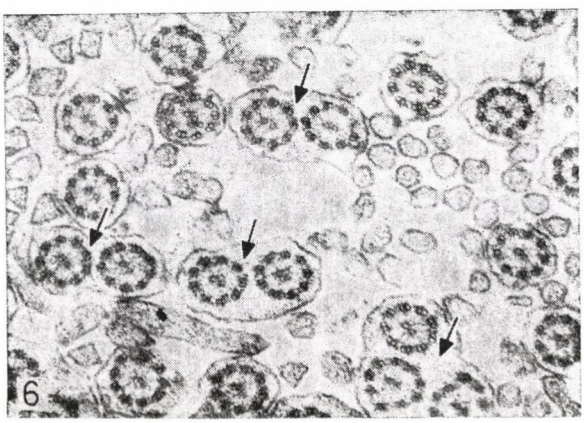
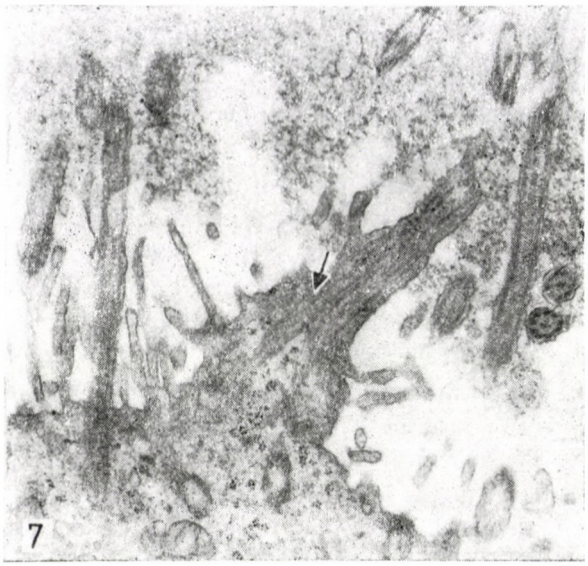


FIG. 5. Cross-sectional profile of a group of cilia. Some cilia show a destroyed outer membrane but maintained microtubuli (↓). Magnification, 1:30 000
FIG. 6. Fusioned cilia ↓ (cross section). Magnification, 1:54 000



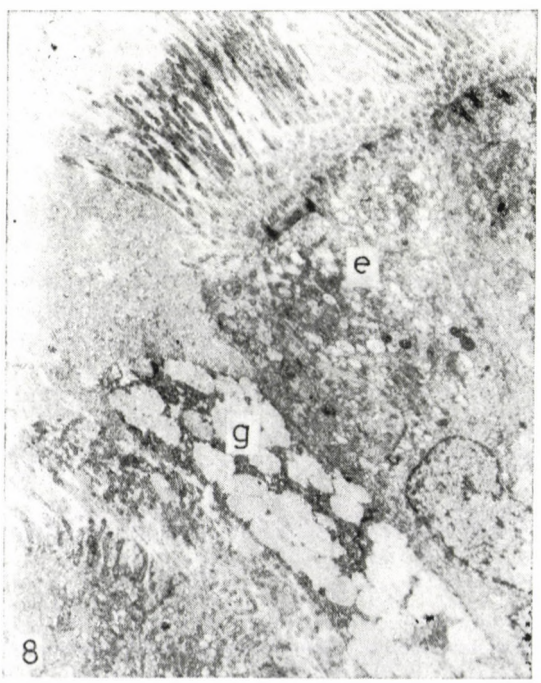


FIG. 7. Longitudinal section of a fusioned cilium (↓). Magnification, 1:28 000
FIG. 8. Goblet cell (g) among vacuolized ciliary epithelia (e). Magnification, 1:5200

chronic inflammation of the bronchial mucosa.

The ultrastructural findings seemed to be more constant. They were not correlated to the above-mentioned signs of inflammation such as the bronchoscopic findings of secretions and the light microscopic changes. These ultrastructural findings might be in correlation with the severity of the underlying disease, and so of prognostic value. This hypothesis should be examined in a group of patients with a variability of the Shwachman score higher than we had in our material. Since the detection of ultrastructural anomalies of cilia in Kartagener syndrome, there have been several investigations into the problem [1, 12, 16, 18, 19, etc.]. Differentiation between the primary ciliary insufficiency (mutated cilia, the immotile cilia syndrome or primary ciliary dyskinesis) and acquired ciliary defects is not always clear-cut. The main differences seem to be that the defects are specific in the first case, and restricted to a certain part of the ciliary apparatus (dynein arms, spokes and central sheath, central microtubules) in the second case. In the opposite acquired defects, the ciliated cells are non-specific and pleiotropic and they frequently involve swelling, fusing and shedding of cilia. Loss of the entire ciliated cell is even more common. An increased occurrence of internalized cilia or cilia with disorganized axononemes is also frequent [1]. Applying these criteria to our results, we are convinced that all ultrastructural changes in CF are of the secondary type, as stated also by others [12, 16, 19]. The acquired ciliary defects may be due to viral infections, infection by mycoplasma or bordetella, a cilio-toxic substance of Pseudomonas aeruginosa, proteolytic substances released from leukocytes in the bronchial secretion, inhalation of penicillin derivatives or mucolytic agents, and allergic reactions. In CF it is more likely that leukocytic enzymes, or drugs used for treatment may play a role in the aetiology of ciliary alterations. In our patients no certain differences could be seen between children who had been treated with mucolytic inhalations or by orally administered mucolytics before the examination.

Mucociliary clearance was normal or slightly impaired [12, 16], and no metaplasia of the bronchial epithelium[13] has been detected. The diagnostic and prognostic value of the present findings requires further investigations over a longer period.

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