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SURFACE MORPHOLOGY OF HUMAN AIRWAY MUCOSA : NORMAL, CARCINOMA OR CYSTIC FIBROSIS

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Abstract

The study presents preliminary qualitative findings of an investigation of grossly normal main and lobar bronchi at sites distant to well circumscribed tumour (n=15), adjacent to tumour (n=5) or of airways obtained during heart/lung transplantation in patients with cystic fibrosis (CF, n=3). In the normal airways the surface epithelium was on average 50 μm thick, pseudo-stratified and rested on a roughly contoured basement membrane. A variety of cell types were identified although many were obscured by a dense covering of cilia, occasionally interrupted by foci of squamous metaplasia. Submucosal gland structure was observed in chance vertical fractures of the airway wall. Tissue adjacent to tumour showed sloughing, squamous metaplasia, pleomorphism and cell surface projections of stubby microvilli or tortuous microridges. The surface morphology of the three CF patients showed no feature unique to the condition, albeit secretions were found adherent to surface lining associated with isolated bacteria and groups of free cells (probably lymphocytes). In each of the three cases the epithelial surface was densely ciliated, interspersed with mucous (i.e., goblet) cells. Submucosal gland collecting ducts had dilated lumena.

KEY WORDS: Airways, bronchi, epithelium, submucosal glands, carcinoma, cystic fibrosis, scanning microscopy.

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Introduction

The light and transmission electron microscopic (TEM) structure of the human bronchus has been described in some detail (Rhodin 1966, McDowell et al 1978, Jeffery 1987) and differences between species have been reviewed (Breeze & Wheeldon 1977, Jeffery 1983). Whilst scanning electron microscopic (SEM) techniques have been applied to the airways of several animal species including man (Motta et al 1977, Wang & Thurlbeck 1970), there is still a paucity of information on the SEM structure of the bronchi of the human lung in health and disease (Sturgess 1982, Simel et al 1984). The emphasis of the present paper is on preliminary qualitative findings of an investigation of grossly normal airways at sites distant to well circumscribed tumour, adjacent to tumour or of airways obtained during heart/lung transplantation in the cases of two young patients with cystic fibrosis. The present paper is preliminary to a larger one which will examine the SEM, TEM and freeze fracture features of extrapulmonary conducting airways.

Materials and Methods

Main or lobar bronchi were obtained within minutes from 15 patients (Table 1) following lung or lobe resection for carcinoma (Ca) (patients 1-14) one of which had bronchiectasis (patient 14) and from 3 patients undergoing heart/lung transplant for cystic fibrosis (CF) (patients 15-17). Grossly normal airways (2-5 cm from tumour or adjacent lobe in case of pneumonectomy) were dissected free of lung and 1-1.5 cm rings were halved by mid sagittal section. Each piece was fixed and stored at 4°C in 3% glutaraldehyde (0.1 M cacodylate buffer pH 7.2) prior to dehydration in graded methanols and transfer to acetone. Acetone was substituted by liquid CO_2 and each piece then critical point dried (Polaron apparatus), mounted with Araldite on aluminium stubs and sputter coated with gold in an atmosphere of argon (Polaron). All specimens were examined using a Philips SEM 501B at 15kV.

Table 1. Summary of Patient Data

Patient	Sex	Age	Diagnosis (Differentiation)
1	M	46	Small cell Ca[1](anaplastic)
2	F	79	Malignant lymphoma
3	M	59	Squamous cell Ca(poor)
4	M	60	Large cell Ca (anaplastic)
5	F	69	Adeno Ca (poor)
6	M	56	Adeno Ca(moderate)
7	F	59	Squamous Ca (poor)
8	M	73	Large cell Ca (anaplastic)
9	F	75	Adeno Ca (moderate/poor)
10	F	32	Adeno Ca(well, broncho-alveolar)
11	M	70	Squamous Ca (poor)
12	F	55	Adeno Ca (poor)
13	F	64	Adeno Ca (moderate Clara/mucous)
14	M	63	Bronchiectasis[2]
15	M	18	Cystic fibrosis[3]
16	F	18	Cystic fibrosis[3]
17	F	31	Cystic fibrosis[3]

1. Ca = carcinoma

2. Cytology performed elsewhere was positive for adenocarcinoma,histology following lobectomy showed bronchiectasis without evidence of tumour.

3. CF tissue was obtained at transplant, all other tissue was by surgical resection

Results

Grossly normal airways

Surface morphology was in general well preserved. The surface epithelium when seen 'on edge' was about 50 um thick and densely ciliated (Fig 1). The pseudostratified structure was apparent as was contact with its roughly contoured basement membrane (Fig. 2). Ciliated cells (referred to as type I cells - see Jeffery et al, this volume for description of cell types) showed the characteristic tuft of apical cilia with large numbers of intervening microvilli (Fig.3). Occasionally the tips of cilia appeared matted together. Secretory cells (previously referred to as types III & IV) were difficult to see but occasionally were seen between dense fields of cilia (Fig. 4). Foci of squamous metaplasia were seen in many samples situated well away from tumour and surrounded by fields of cilia of normal epithelium. The squamous areas consisted of flat surfaced cells with or without microvilli: when present, microvilli were very dense and contrasted with nearby cilia (Fig. 5). The lumenal surface was interrupted by many slit-like openings of the underlying submucosal glands, often with attached strands of mucus (Fig. 6). Ciliated gland ducts could be seen dipping into the submucosa in fortuitous cuts at right angles to the surface of the airway wall (Fig.7). Gland ducts (Fig.8), mucous tubules filled with secretion (Fig.9) and mucous acini were seen (Fig.10).

Fig 1. Epithelial edge of bronchus showing normal pseudostratified structure and densely ciliated surface covering. Beneath are the basal and reticular laminae (r) of the epithelial basement membrane. Patient 2. Bar = 50 μm.

Fig 2. Basal (arrows) and 'superficial' cells of the surface epithelium are shown attached to the adjacent roughly contoured basal lamina which has lost its epithelium. Patient 2. Bar = 10 μm.

Fig 3. Ciliated cells on face show the normal tuft of cilia with large numbers of intervening long slender microvilli (arrows). Patient 3. Bar = 2.5 μm.

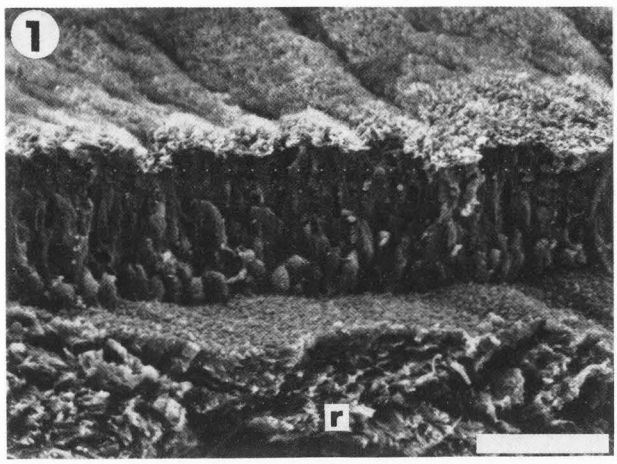
Fig 4. Nonciliated cells of grossly normal mucosa may occasionally bulge into the airway lumen sufficiently to be seen between the dense ciliary covering. Patient 2. Bar = 5 μm.

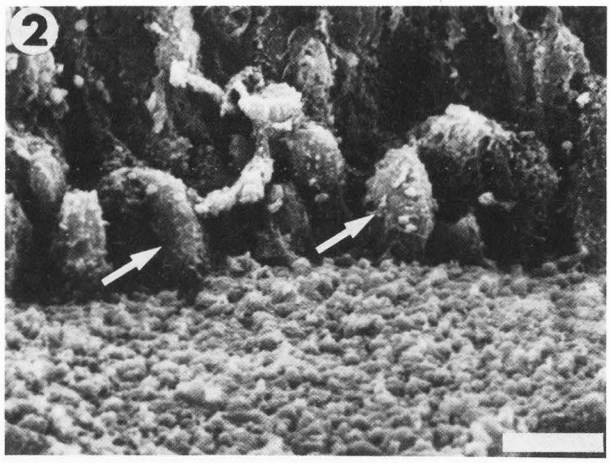
Invasive carcinoma

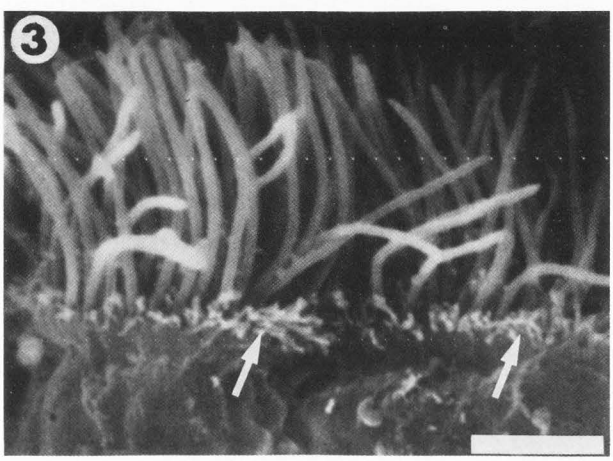
In five patients (patients 9-13) tissue was also sampled at the point of transition between grossly normal tissue and tumour. Histopathology of tissue sections through tumour showed one was a poorly differentiated squamous tumour and the remaining four were adenocarcinomas of the following 4 sub-types: poorly differentiated, moderately differentiated, mixed Clara and mucous cell and one of bronchoalveolar pattern of the mucus-secreting type. The areas surrounding the squamous tumour frequently showed sloughing of epithelial cells and squamous metaplasia with smooth surfaced cells (i.e., cell type VIII). Foci of adenocarcinoma cells were bulging into the lumen of otherwise normal areas of ciliated surface (Fig.11). Tumour cells were enlarged but very variable in size and had surfaces covered by either a dense pattern of short stubby microvilli or slender tortuous microridges (Fig.12).

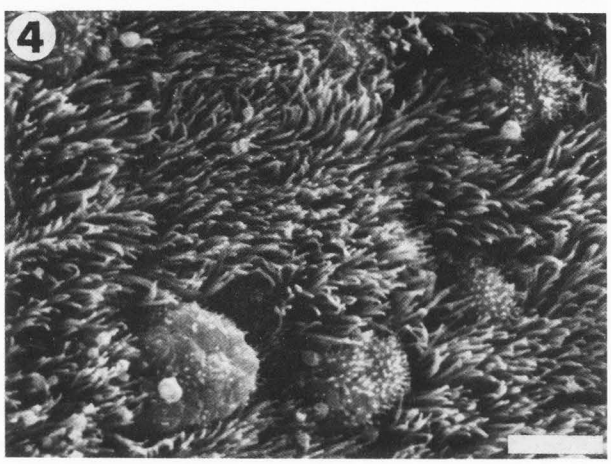
Cystic Fibrosis

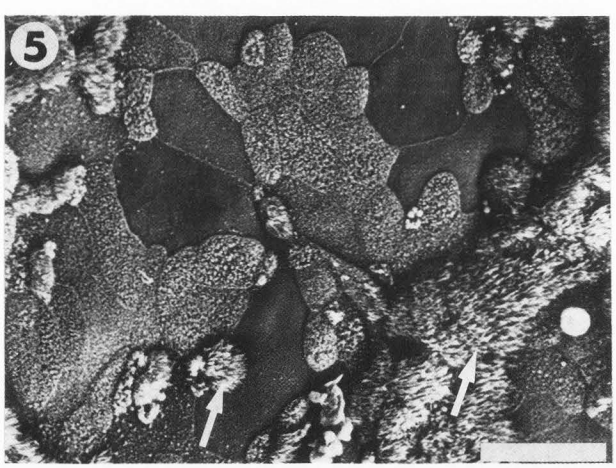
On resection the airways of patients 15 and 16 but not 17 were filled with purulent secretions. Airway surfaces had to be gently washed free of excessive secretions for fixation and processing. Examination by SEM frequently showed secretions still adherent to the tips of cilia (Fig.13). Bacteria were infrequently seen associated with sheets of secretion (Fig.14) while an abundance of cells (probably lymphocytes) were found free or entangled in secretions in the airway lumen (Fig.15). The epithelial surface was densely covered with cilia in each case (Fig.16) and epithelial mucous (i.e., goblet) cells were frequently seen at the cut edge (Fig.17). Foci of squamous metaplasia and cell sloughing were seen: in the former there were often signs of ciliary regeneration. Slit-like submucosal gland ducts were frequent and chance cuts across ciliated and collecting duct areas of the submucosal glands showed the latter had dilated lumena (Fig.18).











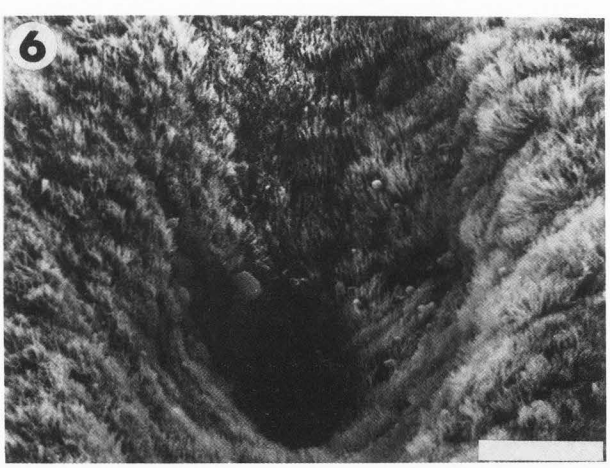


Fig 5. Squamous areas were often seen in otherwise normal epithelium, some with sparse microvilli which contrasted with adjacent cells having either dense covering of microvilli or others with cilia (arrows). Patient 3. Bar = 25 μm.

Fig 6. Normal slit-shaped openings of submucosal glands showing a richly ciliated ductal region. Patient 9. Bar = 25 μm.

Discussion

Grossly Normal Mucosa

The extensive coverage of mucosa by cilia in human airways contrasts with the approximately 50% covering shown in the specific pathogen free (SPF) rat (Jeffery et al - this volume) but is similar to that seen in several other mammalian species (Sleigh 1977). The degree of ciliation makes it difficult to see the intervening non-ciliated cells whose apices only rarely protrude beyond the ciliary fringe. Chance cuts across the edge of the epithelium do, however, show that there are large numbers of secretory cells which, from the surface, go unseen, each with abundant intracellular secretory granules (i.e., cell type IV). The same cuts show the pseudostratified arrangement of the epithelium which is at least twice as thick as that of the SPF rat trachea. Foci of squamous metaplasia at sites away from the tumour emphasise the multifocal nature of malignant disease and the widespread potential for development of pre-malignant and malignant change (Spencer 1985).

The TEM ultrastructure of submucosal glands has been previously examined in 19 human cases and a three dimensional reconstruction made in at least one (Meyrick et al 1969, 1970). The division of a single gland unit into ciliated, collecting and secretory tubules is illustrated in the present SEM study.

Carcinoma

Areas of mucosa immediately adjacent to tumour show the pleomorphism described by classic histopathology. The enlarged and bulging cells of the adenocarcinomas give a three dimensional view which emphasises the malignant nature of the cells in a way more dramatic than that seen by examination of sections. A variety of surface microvilli have been described on the surface of pulmonary adenocarcinomas of differing degrees of differentiation (Lupulescu & Boyd 1972, Rainio et al 1982, Rainio 1983, Henderson et al 1986). The presence of microridges has been suggested as a marker of moderate to well differentiated tumours (Rainio et al 1982). Surface microridges have, however, also been described on airway epithelial cells in non-malignant disease such as CF (Simel et al 1984) and their significance is, as yet, unclear.

Cystic fibrosis (CF)

SEM studies of the surface structure of airways obtained during post-mortem from patients dying of CF have been reported by Sturgess (1982) and by Simel et al (1984). The present paper, whilst only on 3 cases, gives information obtained from studying transplant tissue fixed at the time of removal from the patient and examines also the cut edges for submucosal gland structure. The tenacity of the mucus is a characteristic of the condition which affects glands and epithelia throughout the body (see Mangos & Boyd 1984). Bacterial infection (particularly by Pseudomonas aeruginosa) is also a feature and the rod forms seen in the present study are likely to be the organism responsible for the purulence observed on dissection. In agreement with Simel et al (1984) we also found surprisingly few bacteria attached to the surface epithelium. We believe most are washed away with the mucus during rinsing, fixation and processing. The free inflammatory cells seen are the right size and morphology for the lymphocytic infiltrate associated with hyperplasia of bronchial lymphoid tissue reported in the light microscopic studies of Tomashefski et al (1983). Another histopathological feature reported by the same authors is the distension of submucosal gland lumena by mucus. The present study confirms the distension of the collecting duct area and gives a novel three dimensional view of gland micro-structure. The extensive covering of the surface by cilia of normal structure is in accord with the observations of Simel et al (1984) and of interest in the light of reports showing reduced mucociliary clearance in CF (Wood et al 1975, Yeates et al 1976, Kollberg et al 1978). In collaboration with others (Steinfort et al - unpublished) we have shown that CF cilia, taken from tissue adjacent to that examined by scanning microscopy and washed free of their bacteria and secretions by culture media, will beat with a frequency within the normal range. Wilson et al (1985, 1987) has shown that bacterial products from P. Pseudomonas aeruginosa and Haemophilus influenzae, but not Staphylococcus aureus, are powerful inhibitors of ciliary beat. Our studies show that if these products can be

Fig 7. Mucosal edge from a grossly normal area showing ciliated duct region (arrow) dipping through the mucosa to connect with deeper mucous tubules. Airway lumen (L). Patient 9. Bar = 50 μm.

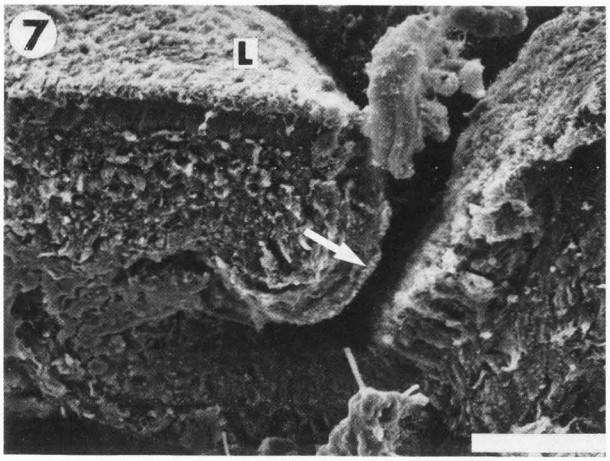
Fig 8. Ductal tubule in cut edge of normal submucosa. The internal surface structure of individual lining cells can be seen (arrow) and the tubule is surrounded by strands of collagen. Patient 5. Bar = 25 μm.

Fig 9. Mucous tubule whose cut lumen is spilling secretion. Tubule surrounded by collagen sheath. Patient 14. Bar = 10 μm.

Fig 10. Mucous acinus with wide internal lumen surrounded by secretory cells filled with discrete secretory granules (arrows). Patient 5. Bar = 10μm.

Fig 11. Area adjacent to adenocarcinoma (moderate/poor differentiation) showing foci of enlarged cells of variable size bulging into the airway lumen of otherwise normal ciliated epithelium. Patient 9. Bar = 100 μm.

Fig 12. Higher magnification of the lumenal surface of tumour cells adjacent to a broncho-alveolar tumour (mucus-secreting type) show either a dense pattern of short stubby microvilli or slender tortuous micro-ridges. Arrows show cell borders of a single cell. Patient 10. Bar = 2.5 μm.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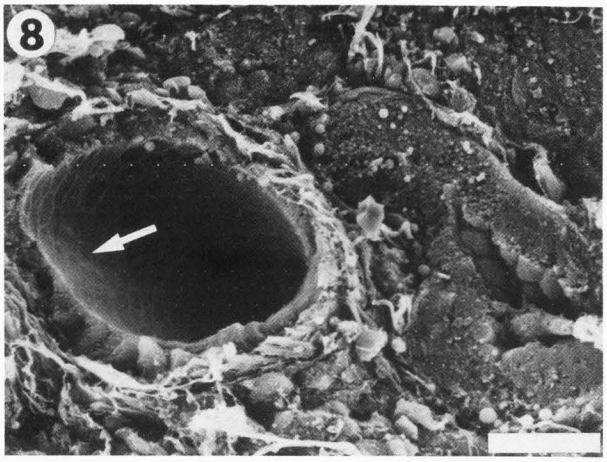
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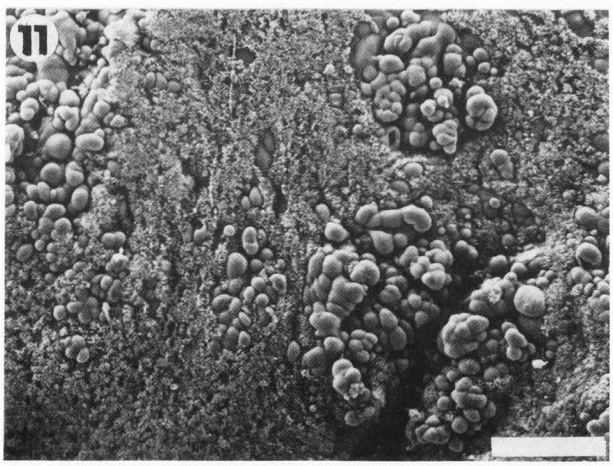
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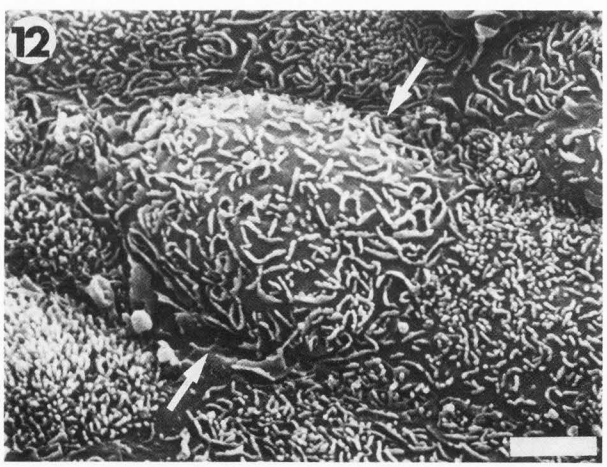
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neutralized then the cilia in CF, which are present in large numbers and structurally intact, are able to re-commence beating to clear the excessive secretions from airways whose function is otherwise compromised.

Acknowledgements

We are particularly grateful to Miss Fiona Nelson for her technical help and to Mrs Jenny Billingham for her patience and accuracy in typing the manuscript. We thank the transplant teams at Papworth (Cambridge) and Harefield (Middlesex) hospitals for their helpful cooperation and Dr. E. Sheffield in our department for assisting with the surgically resected specimens.

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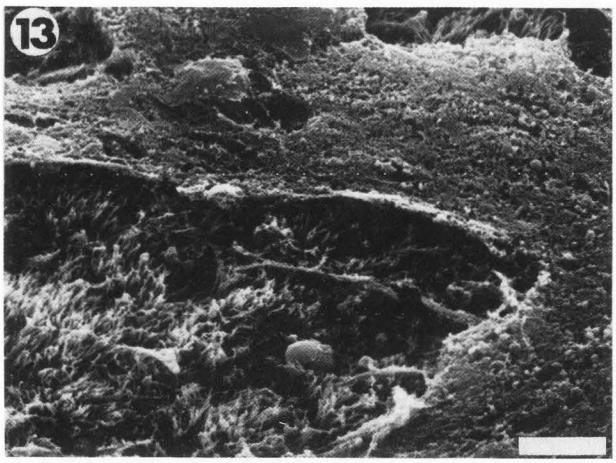
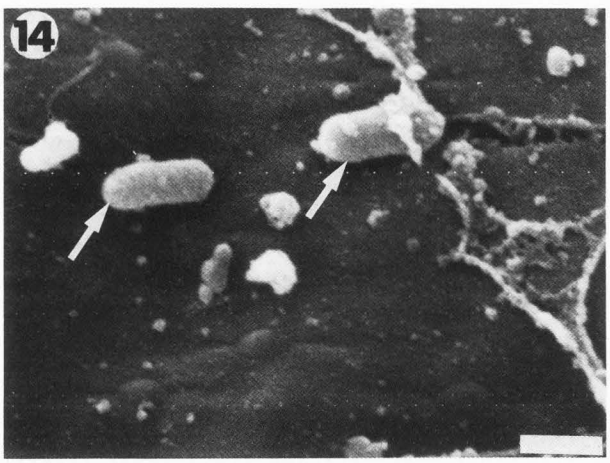
Fig 13. Airway surface from a patient with cystic fibrosis (CF) illustrating secretions, which remain after processing, adherent to ciliary tips. Patient 15. Bar = 10 μm.

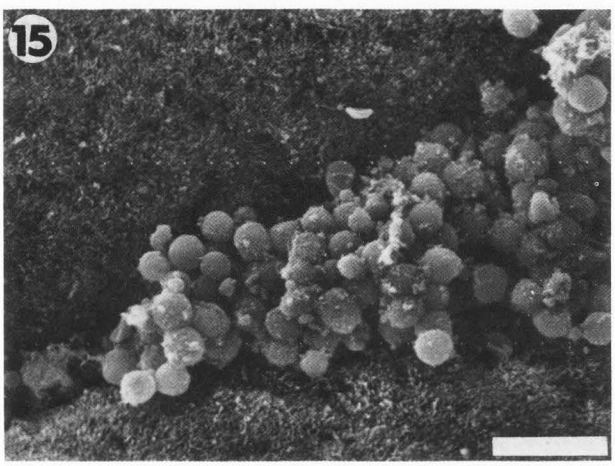
Fig 14. Two rod forms (arrows) on a sheet of airway secretion in a patient with CF. They are the size and shape of laboratory cultured Pseudomonas aeruginosa. Patient 15. Bar = 1 μm.

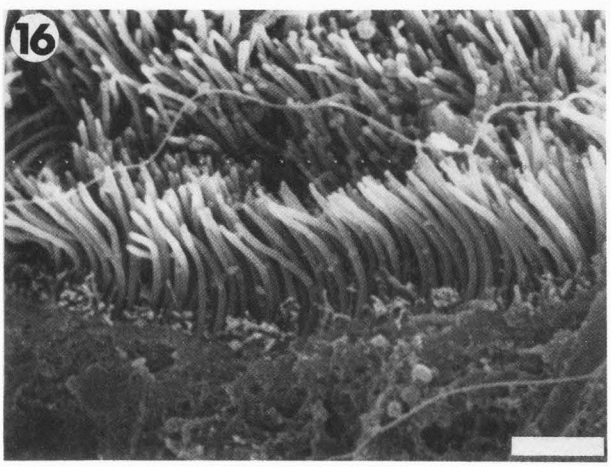
Fig 15. One of many aggregations of free cells, probably lymphocytes, found on the ciliated surface of a CF airway. Patient 16. Bar = 25 μm.

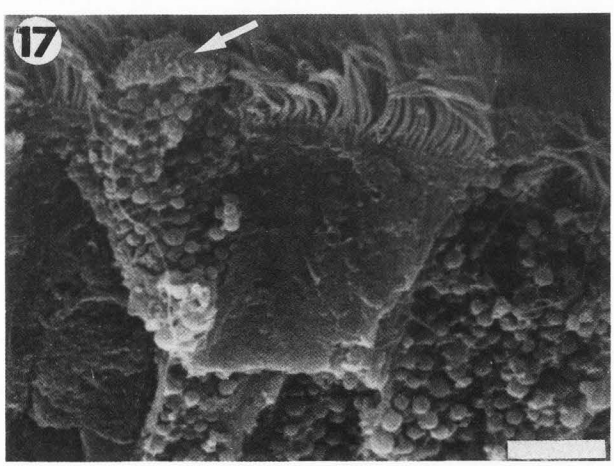
Fig 16. CF airways were densely covered by cilia, of normal structure and density. Cut edge showing cilia and intervening microvilli. Patient 15 . Bar =2.5 μm.











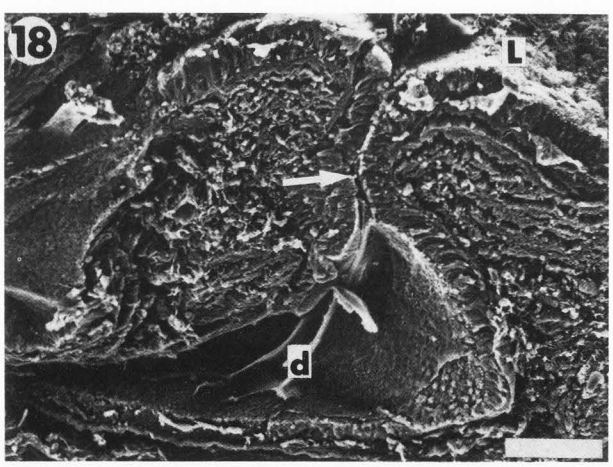


Fig 17. From the surface, epithelial mucous cells were difficult to see but cut on edge there were many, each filled with individual secretory granules and covered by an apical microvillus cap (arrow). Patient 16. Bar = 5 μm.

Fig 18. Random cuts across submucosal gland ducts showed that collecting ducts (d) in CF bronchi had dilated lumena. Ciliated duct (arrow) leading to airway lumen (L). Patient 16. Bar = 100 μm.

Discussion with Reviewers

L. de Saint-Georges: Did the physiological approach (studying ciliary beat frequency) concern human or rat?
Authors: Studies of ciliary beat frequency were conducted in parallel, using human tissue adjacent to that studied by S.E.M.

L. de Saint-Georges: How can such a conclusion be drawn from an essentially descriptive work? SEM study alone is not sufficient to know whether a structure is intact or not.
Authors: Transmission microscopy of the same tissue (not reported herein) showed that the cilia were of normal structure either in longitudinal or transverse section and that the ciliary hooks or "crown" were present. The combination of scanning, transmission and parallel functional studies do indicate, we feel,that the cilia in CF are not irreversibly damaged.

B. Afzelius: One way of getting rid of the mucus blanket before examination with the scanning electron microscope is to let the cilia transport off the mucus before the biopsy is fixed. Does this method work also in cases of cystic fibrosis?
Authors: On dissection the airways of CF patients are filled with purulent secretions almost exclusively of multiresistant Pseudomonas aruginosa. This organism is known to release cilotoxic factors (Wilson R et al 1987, text ref.) which probably explains why the novel method suggested does not work well with CF airways. We do know, however, that after rinsing thoroughly with culture media the CF cilia beat with a frequency within the normal range (unpublished data).

B. Afzelius: What are the cells in figure 5 that are characterized by many long microvilli? Brush cells are not described from human airway mucosa.
Authors: The cells with long slender microvilli are most probably ciliated cells having lost or in the process of regenerating their normal complement of cilia. The "brush border" of brush cells described in other species is quite distinct in having fewer and thicker projections which have never, in the authors' opinion, been convincingly described in man.

B. Afzelius: The subjective impression is gained from inspecting your micrographs that the goblet (mucus) cells produce the regular mucus blanket that is active in the mucociliary clearance of the airways, whereas the larger submucosal glands produce larger blobs of mucus that may be cleared in the productive coughing. The submucosal glands would then be productive only in bronchitis. Is there any evidence for such an interpretation?
Authors: The idea is an interesting one which, as yet, has no supportive evidence. A third source of mucosubstance rich in glycosaminoglycans which probably forms the bulk of normal secretions is the glycocalyces of many cell types including the ciliated cell.

G. Roomans: It has been suggested that in studies of CF airways one should use tissue of patients with other chronic airway diseases (chronic bronchitis, bronchiectasis) as a control group, rather than "normal" tissue. What is your opinion?
Authors: Yes, resected bronchiectatic tissue would be the most suitable control tissue to use. Only one of our cases was bronchiectatic. We have used bronchiectatic tissue to control our studies of autonomic receptors and lung innervation but it has proved difficult to recover the large central airways required in sufficient quantity for the present study. Our efforts are continuing.

G. Roomans: Since it is assumed that the basic defect in CF is related to the error in epithelial ion and water transport, it would be interesting to know whether the epithelial cells of CF airways were enlarged in comparison to control tissue. Do you have any data on this?
Authors: The suggestion is a very interesting one. We did not observe undue bulging of the apical surfaces of cells in our scanning electron microscopic study. Our parallel transmission EM and culture studies should allow such an analysis to be done.