The Journal of Laryngology and Otology
December 1994, Vol. **108**, pp. 1024–1030

Surface morphology of middle ear epithelium in chronic ear disease

A. M. AGIUS*, M. WAKE†, A. L. PAHOR*, L. A. SMALLMAN‡

Abstract

The ultrastructural details of normal middle ear mucosa have been reported in detail in the literature. This study was aimed at investigating the superficial structure of middle ear epithelium with the scanning electron microscope in patients with persistent otitis media with effusion (OME) and chronic suppurative otitis media (CSOM), especially in the light of the authors' recent findings regarding decreased ciliary beat frequency (CBF) in patients exposed to tobacco smoke.

Mucosal biopsies were taken from the anterior mesotympanum in four patients with OME and another four patients with CSOM. There was no significant abnormality of cellular surface structure in patients with chronic middle ear disease. Furthermore, no significant difference was observed between smoke exposed and nonsmoke exposed patients. It is concluded that the effect of tobacco smoke on CBF is functional and not related to any anatomical abnormality observed by scanning electron microscopy (SEM).

Key words: Otitis media with effusion; Otitis media, chronic suppurative; Microscopy, electron, scanning

Introduction

Sadé (1967) was the first to recognize the mucociliary system in the human middle ear as a functional entity when he observed that small particles placed on the middle ear mucosa through a perforation in the tympanic membrane travelled towards the eustachian tube.

The human middle ear cavity is lined by ciliated epithelium in its antero-inferior half to two-thirds, which extends from the eustachian tube to the hypotympanum, promontory and anterior epitympanum (Lim and Hussl, 1969). Ciliated epithelium has been shown to be morphologically identical to other types of respiratory lining (Kawabata and Paparella, 1969). Kawabata and Paparella (1969) were the first to describe the fine detail of human middle ear mucosa from biopsies which they took from otosclerotic patients. Shimada and Lim (1972) later showed three main ciliated cell pathways from the hypotympanum, promontory and epitympanum to the eustachian tube orifice and that the distribution of secretory cells closely correlated with that of the ciliated cells (Lim and Shimada, 1973) thus together constituting the mucociliary transportation system. Transmission electron microscopy (TEM) of middle ear mucosa in normal subjects demonstrated the presence of several surface epithelial cell types, including nonciliated cells, ciliated cells and secretory cells (Lim and Hussl, 1969). Nonciliated cells possess microvilli on their free surface – it has been suggested that these structures are involved in the transport of water out of the middle ear cavity into the underlying lymphatic spaces, thus contributing to the viscosity of middle ear effusion in OME (Hilding and Heywood, 1971). Secretory cells consist of two types: goblet cells (which on TEM contain light-coloured secretory granules) and intermediary cells (which contain secretory granules of varying density). Tos (1980) has shown goblet cells to be increased in density in chronic secretory otitis.

In a systematic study of temporal bones, epithelium has been shown to undergo a transition postero-superiorly so that the antrum and mastoid air cells are lined by a single layer of columnar, cuboidal, or squamous cells (Lim and Hussl, 1969; Shimada and Lim, 1972).

Our study aimed to systematically examine the ultrastructure of ciliated middle ear mucosa in patients with chronic OME and CSOM by sampling a standard site, i.e. the anterior mesotympanum. This site was considered more likely to yield a satisfactory sample of ciliated cells and was the site of choice when sampling individuals with OME who were undergoing myringotomy.

A significant reduction in ciliary beat frequency (CBF) has been observed in middle ear mucosa of individuals exposed to tobacco smoke (Agius *et al.*, in press). The possibility of a morphological explanation for this reduction in CBF was therefore investigated. This study further sought to compare the findings in smokers, or those passively exposed to tobacco smoke, in contrast to individuals not so exposed.

Methods and materials

Eight patients were entered into the study, four with

From the Departments of Otolaryngology, Dudley Road Hospital*, Birmingham, and the Royal Hospital†, Wolverhampton, and the Department of Pathology‡, The Medical School, University of Birmingham.
Accepted for publication: 30 July 1994.

persistent OME and four with CSOM. Two patients from each group gave a history of smoking or of habitual passive exposure to tobacco smoke.

A standard proforma was adopted. This included details of symptomatology, duration, previous otological treatment, and history of atopy. A history of exposure to tobacco smoke, both active and passive, was also taken. No attempt was made to quantify the degree of smoke exposure and patients were only classified as smoke exposed or nonsmoke exposed.

All OME patients satisfied the following criteria over a time-frame of at least six months: (1) persistently abnormal tympanic membrane (including colour changes, loss of light reflex, areas of retraction, presence of bubbles in the middle ear); (2) at least two abnormal tympanograms (one of which was type B: the other may have been type B or C) carried out at least six months apart; (3) consistently abnormal pure tone audiogram with an average conductive hearing loss of 25 dB or more worked out over three frequencies (0.5, 1.0 and 2.0 kHz).

Following antero-inferior myringotomy and removal of middle ear effusion by microsuction, biopsies of the middle ear mucosa were taken from the anterior mesotympanum by means of cupped forceps introduced through the incision.

CSOM patients all gave a history of recurrent otorrhoea and hearing loss for at least six months duration. Biopsies were all taken from the anterior mesotympanum in these patients.

All samples were fixed immediately in 2.5 per cent glutaraldehyde in phosphate buffer (pH 7.4) and allowed to stand overnight at 4°C. They were then dehydrated through a graded acetone series using 50, 70, 90, 95 and finally 100 per cent dried acetone. Critical point drying was carried out in an Emscope CPD 750 critical point drier and the samples were mounted onto copper stubs using double-sided carbon adhesive tape and sputter-coated with platinum in an Emscope SC 500 sputter coater. They were then examined in a Jeol 100CX II electron microscope. Linear measurements were calculated according to scale while a grid method was used to calculate surface area, again based on scale.

Approval for this study was granted by the West Birmingham District Medical Ethical Committee as it involved invasive tissue sampling not routinely carried out during operative procedures.

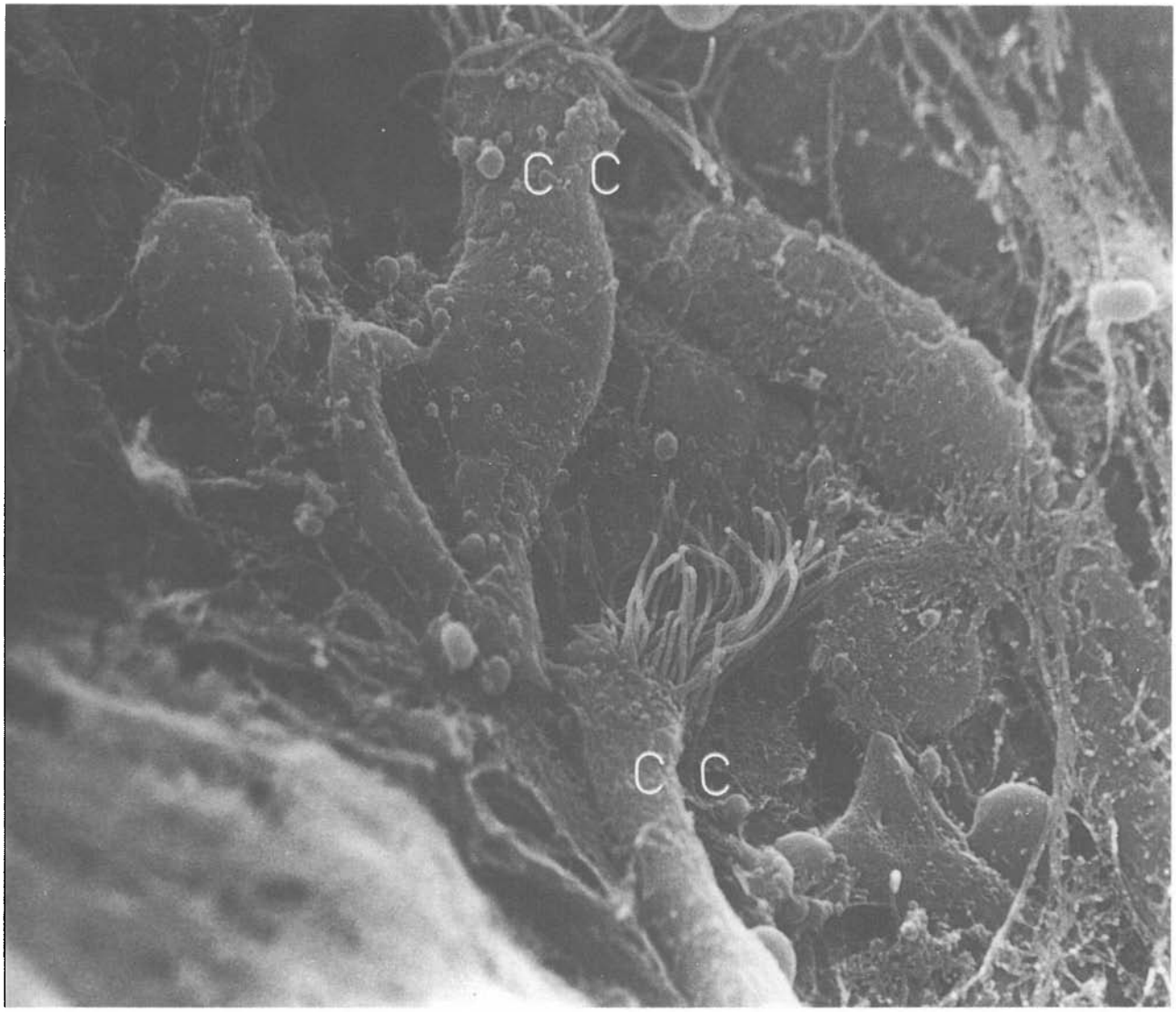

FIG. 1
SEM showing ciliated epithelial cells (CC) (× 5100).

https://doi.org/10.1017/S0022215100128828 Published online by Cambridge University Press

Results

Eight patients were studied; four were males and four females. Their ages ranged from nine to 43 years, with a mean of 19.2 years. All the OME patients had at least one previous ventilation tube insertion (range one–four previous ventilation tubes). Three patients were effusion positive at operation, and had a mucoid effusion. All the CSOM patients had dry ears at operation. One CSOM sample was unsuitable for analysis as no mucosal surface could be identified on scanning the fixed biopsy fragment.

All other biopsies were suitable for examination. Samples were scanned at magnifications ranging from × 770 to × 96 000. The following epithelial cells were identified.

Ciliated cells

These cells were 22–30 μm in length and between 2–5 μm in width (Figure 1). Cilia varied in length between 3 and 7 μm and their diameter ranged from 0.16 to 0.19 μm. The ciliated area contributed by an individual ciliated cell varied between 10.5 and 14.7 μm^2. Cilia presented a notched appearance just proximal to their tip which was slightly bulbous (Figure 2). Their external surface was roughened; this could have been surface glycocalyx. Ciliary crowns, described in TEM sections, were not visible in any of the preparations. Mucosal surfaces of area varying between 225 and 775 μm^2 were studied more closely. Ciliated area as a proportion of total surface area varied widely, from 35 to 76 per cent, mean 57 per cent, and there was no significant difference between OME and CSOM patients. Similarly, no significant difference in proportion was observed between those habitually exposed to cigarette smoke and those not so exposed. No abnormal ciliated cells were identified (Figure 3), although cilia seemed to be shorter in comparison to previous findings in normal ears (Shimada and Lim, 1972).

Nonciliated cells

These varied between 18 and 20 μm in length and between 1.8 and 4.3 μm in width (Figure 4) and their epithelial surface was characterized by projecting microvilli (Figure 5). Microvilli were between 0.5 and 0.75 μm in length and 0.13 to 0.15 μm in diameter. Most microvilli

FIG. 2

A closeup view showing the roughening of the cell membrane overlying the cilial surface, probably glycocalyx. The notched cilial ends just distal to their tip are arrowed (× 96 000).

https://doi.org/10.1017/S0022215100128828 Published online by Cambridge University Press

FIG. 3

Ciliated epithelium with intervening nonciliated cells bearing microvilli: a mucus globule (G) is being propelled along by cilia (× 6900).

were straight but some branched. At their free surface, nonciliated cells accounted for an area that ranged between 18.9 and 27.7 μm^2.

Goblet cells

These were identified by the formation of convex droplets of mucus (Figure 6) and exhibited a stoma in the cell membrane representing the site of extrusion of the mucus globule. Globules were smooth or rough and varied in diameter between 0.5 and 2.4 μm. The larger ones bore residual microvilli and seemed to consist of the distal parts of secretory cells, as in apocrine secretion. Goblet cell density varied but they generally tended to occur in densely ciliated regions. A mean value of one cell per 1736 μm^2 of epithelial surface area was calculated (or one goblet cell to 100 epithelial cells). However, as SEM only enabled visualization of actively extruding goblet cells at the particular moment of biopsy, any calculation of goblet cell density could underestimate the true value.

Discussion

TEM studies of middle ear mucosa demonstrated three types of cells: ciliated cells, nonciliated cells and secretory cells (Lim and Hussl, 1969). The last variety includes goblet cells and intermediary cells. Basal cells are also present but for the purposes of this study were not considered.

All biopsies in this study were taken from the anterior mesotympanum where a large number of ciliated cells was expected. One biopsy was not satisfactory because of difficulties with stereoscopic orientation and fixing of tiny samples with the epithelial surface uppermost.

Tos (1980) has shown an increase in both ciliated cells and goblet cells in middle ear biopsies from patients with chronic OME. He noted that the pseudostratified epithelium in the anterior segment of the middle ear was thickened. This was accounted for by basal cell hyperplasia. The middle ear lining in the posterior segment of the tympanic cavity, normally a flat type of epithelium, was transformed into pseudostratified columnar epithelium by basal cell hyperplasia and metaplasia. Our biopsies from the anterior mesotympanum of OME patients show no significant difference in the proportion of ciliated surface areas compared to normal. This finding is repeated in our patients with CSOM. The fine ultrastructural detail

https://doi.org/10.1017/S0022215100128828 Published online by Cambridge University Press

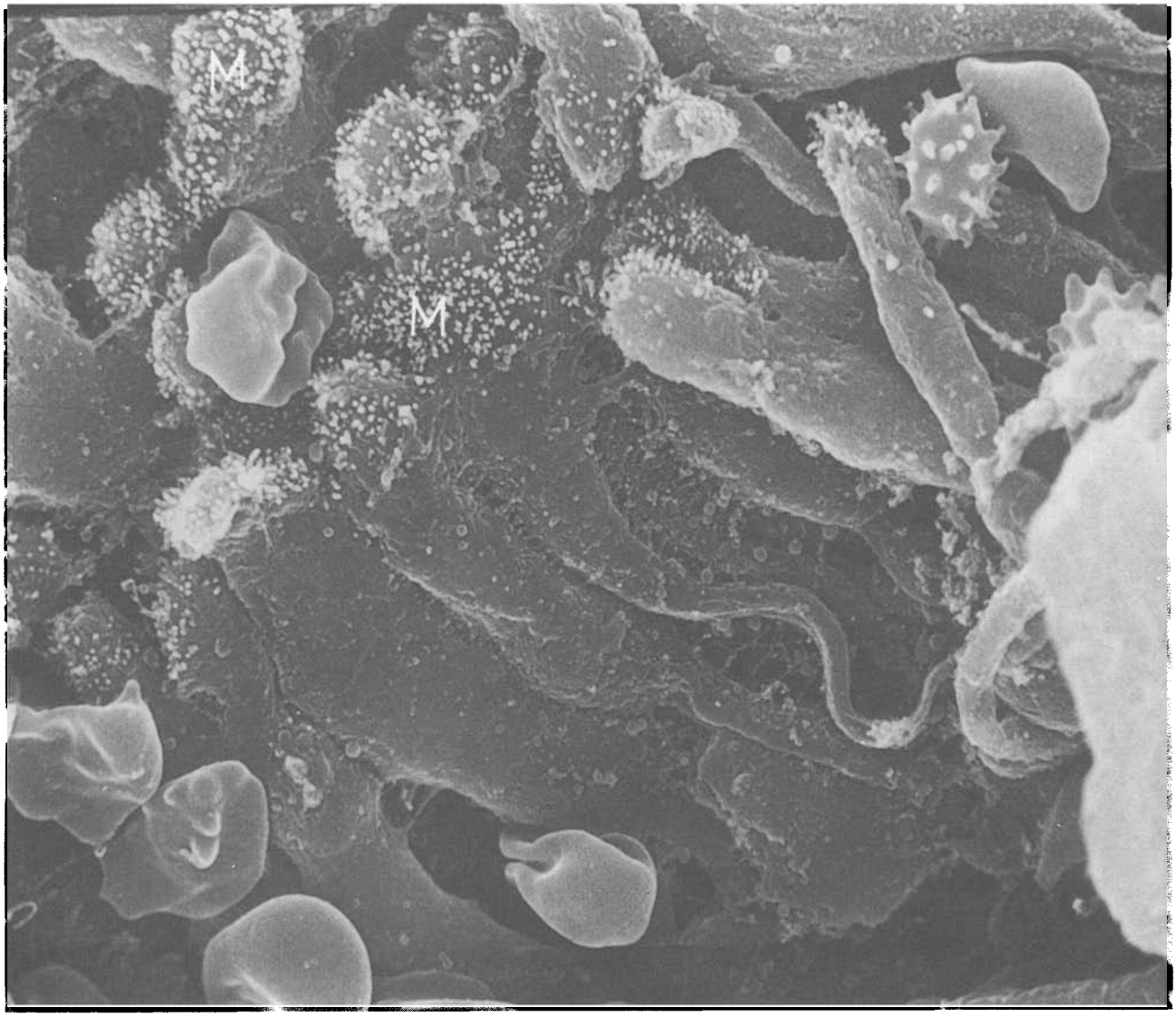

FIG. 4
Nonciliated epithelial cells bearing surface microvilli (M) (× 7500).

of middle ear mucosa in CSOM, however, has not been so well studied as in OME. Lim (1979) estimated that in the anterior mesotympanum 50–80 per cent of cells are ciliated. All our biopsies demonstrated a similar range of ciliated surface area. This implies that OME, CSOM or tobacco smoke does not alter the balance between ciliated and nonciliated cells in this region of the middle ear.

Middle ear inflammation and tobacco smoke may however affect the maturation of cilia. Cilial dimensions in this study were below the average length and width of other cilia recorded elsewhere in the upper respiratory tract. It is of interest that Chang (1957) in a study of human bronchial epithelium noted that smokers tended to have shorter cilia. No ciliary crowns, as visualized by TEM (Davis and Smallman, 1988) were visible at the tips of the cilia. Cilial ends were noted to be rather bulbous, their bulbosity accentuated by a notch just proximal to the tip, and which gave the impression of a hook. It is thought that this structure may aid propulsion of the mucus blanket.

The mucus blanket, arising from the secretions of goblet cells and nonciliated cells, is the second essential component of the mucociliary system, and it has been noted that ciliated and secretory cells occur in clearly defined 'coupled' tracts in the middle ear (Shimada and Lim, 1972). The larger secretory globules bore microvillous structures, showing that whole parts of distal secretory cells were being shed from the lining mucosa.

Goblet cells appear to be rather infrequent in our study of the middle ear mucosa, and although an increase of such cells in tobacco smoke exposed individuals was expected, as occurs in the bronchial tree (Heath and Kay, 1985), this was not borne out by the results. It is possible that goblet cells would be demonstrated more frequently by TEM, as in SEM views, only the ones which are actively extruding at the particular moment of the biopsy would be visible. Nonextruding goblet cells present a surface appearance of microvilli, indistinguishable from other nonciliated cells. It is also known that the mean number of goblet cells in the middle ear mucosa is lower than that in the nose. These considerations might account for our relatively low density of 580 goblet cells per square millimetre, compared to goblet cell density on the inferior turbinate of between 6100 and 12 700 (Proctor, 1982). Tos (1980), in a histological study on middle ear epithelium in patients with chronic OME put goblet cell

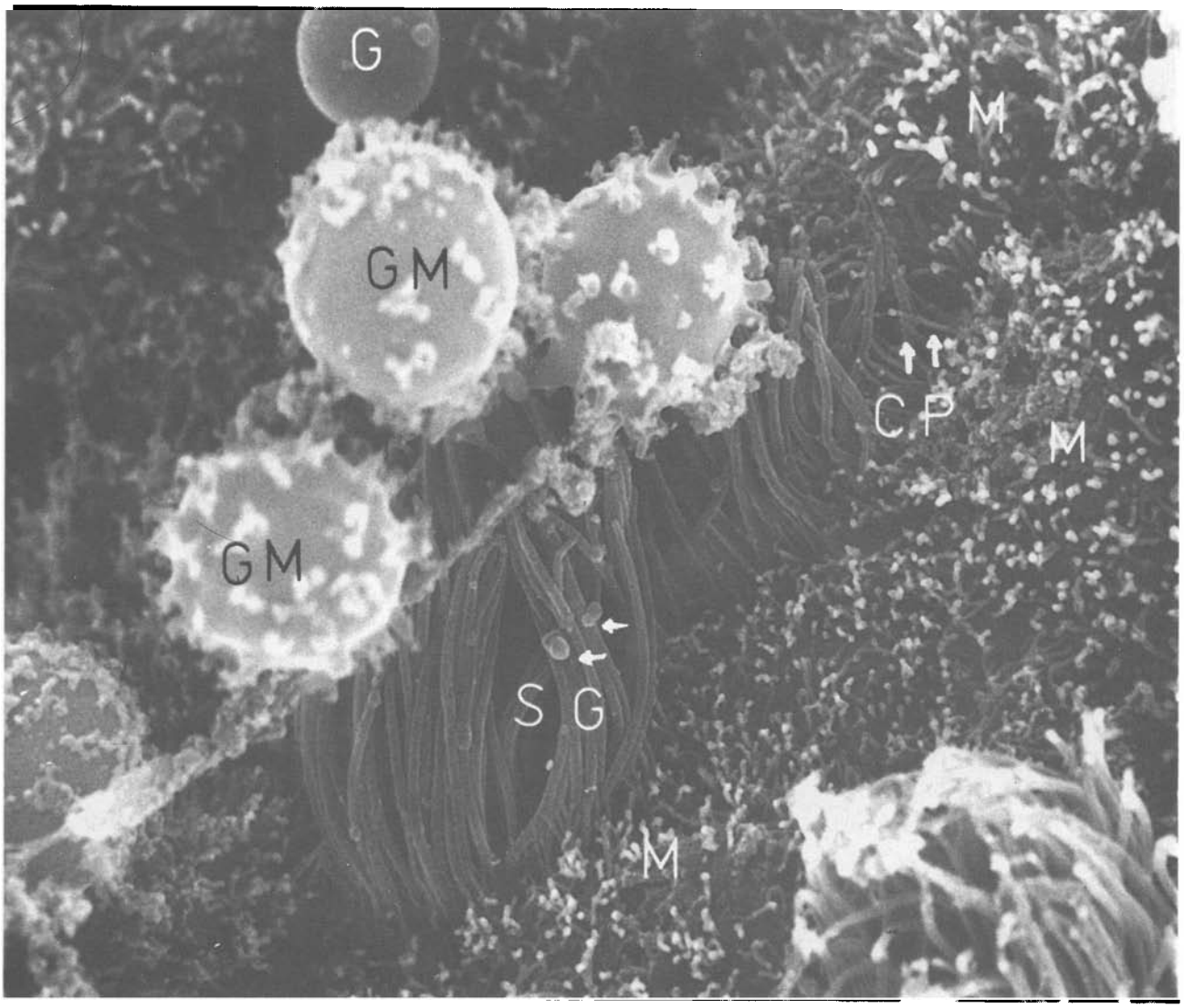

FIG. 5

Ciliated and nonciliated epithelial cells, the latter with surface microvilli (M): fine cytoplasmic projections (CP) may be seen at cellular junctions. Also visible are smooth mucus globules (G) and globules bearing residual microvilli (GM). Secretory granules (SG) are also visible (× 12 300).

density at between 2900 and 14 500 per square millimetre.

Studies by Hentzer (1972 a and b) showed a relative decrease in the number of ciliated cells in middle ear mucosa in OME compared to normal. He also showed alteration of the mucosal lining in OME towards greater secretory activity with ciliated cells losing their cilia and acquiring dark granules in their cytoplasm. Apocrine secretion was demonstrated with discharge of distal parts of cells into the middle ear cavity. A similar mechanism has been described in nasal epithelium (Davis and Smallman, 1988). This would explain our SEM findings of globules bearing microvilli remnants on their surface.

Conclusions

SEM of middle ear mucosa of patients with OME or CSOM showed no structural differences from normal middle ear mucosa. There was no significant morphological difference between those patients actively or passively exposed to tobacco smoke and those not so exposed. This indicates that the reduction in CBF shown by tobacco-exposed individuals is a functional phenomenon as anatomical differences were not demonstrable on SEM. The answer may possibly lie in the action of the metabolites of tobacco smoke on secondary cellular messengers such as cAMP or Ca^{2+} but further work is required in this area.

Acknowledgements

The authors would like to thank the Midland Institute of Otology for financially supporting this work. The help of Mr P. Stanley, scanning electron microscopist at the Department of Physiology, the Medical School, University of Birmingham is gratefully acknowledged. The advice and practical contributions of Mr P. E. Robin and Mr P. J. Dekker, Consultant Otolaryngologists at Dudley Road Hospital, is kindly acknowledged; also their help in allowing their patients to be studied.

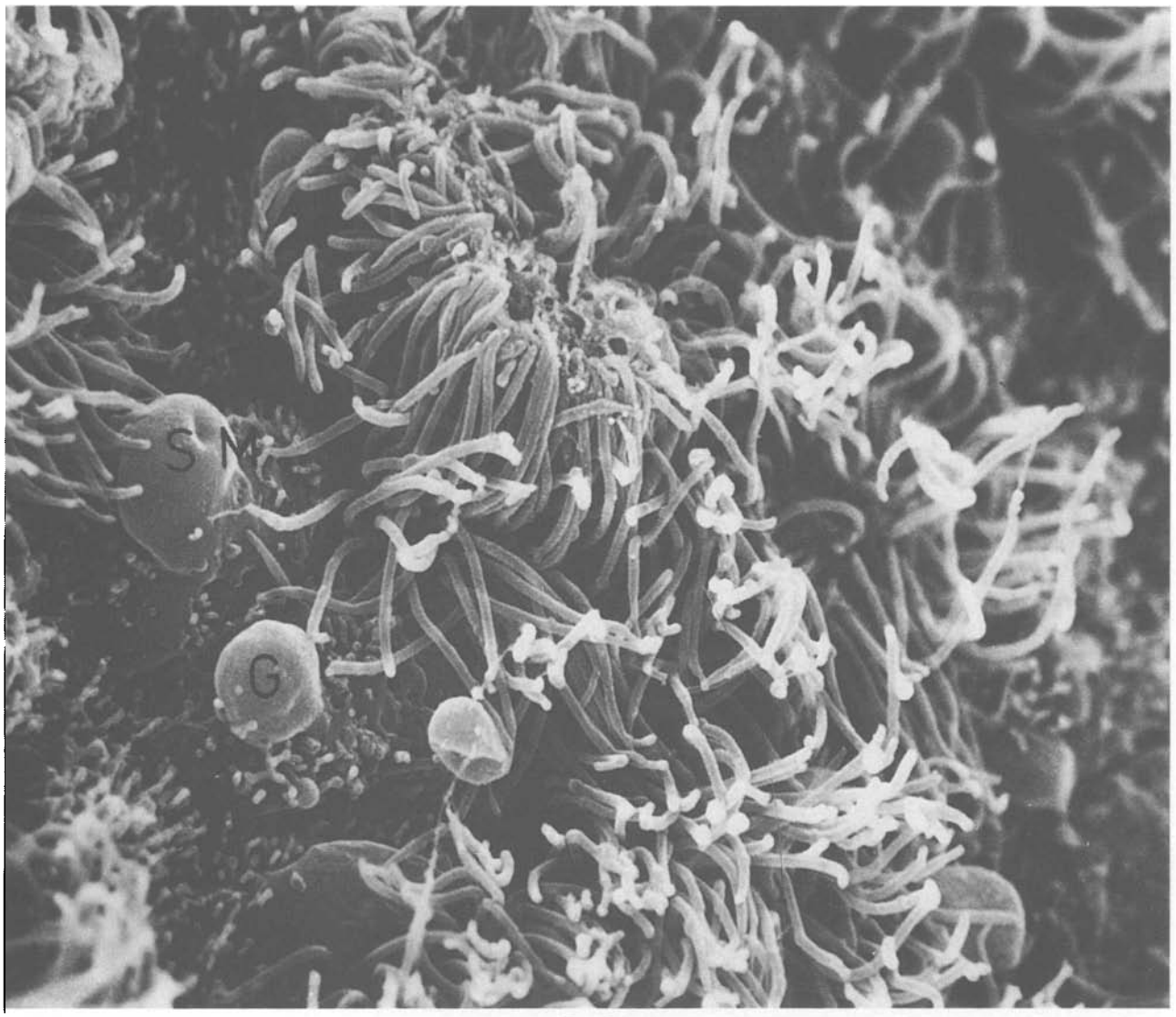

FIG. 6

Active goblet cell showing stoma (SM) from where mucus globule (G) has been extruded. Surface of goblet cell demonstrates microvilli (× 13 100).

References

Agius, A. M., Wake, M., Pahor, A. L., Smallman, L. A. (in press) Smoking and middle ear ciliary beat frequency in otitis media with effusion. *Acta Otolaryngologica* (Stockholm).

Chang, S. C. (1957) Microscopic properties of whole mounts and sections of human bronchial epithelium of smokers and non-smokers. *Cancer* **10:** 1246–1262.

Davis, A. E., Smallman, L. A. (1988) An ultrastructural study of the mucosal surface of the human inferior concha. I. Normal appearances. *Journal of Anatomy* **161:** 61–71.

Heath, D., Kay, J. M. (1985) Respiratory system. In *Muir's Textbook of Pathology.* 12th Edition. (Anderson, J. F., ed.), Edward Arnold, London, p 16.

Hentzer, E. (1972a) Ultrastructure of the middle ear mucosa in secretory otitis media. I: Serous effusion. *Acta Otolaryngologica* **73:** 394–401.

Hentzer, E. (1972b) Ultrastructure of the middle ear mucosa in secretory otitis media. II: Mucous effusion. *Acta Otolaryngologica* **73:** 467–475.

Hilding, D. A., Heywood, P. (1971) Ultrastructure of middle ear mucosa and organization of ciliary matrix. *Annals of Otology* **80:** 306–311.

Kawabata, I., Paparella, M. M. (1969) Ultrastructure of normal human middle ear mucosa. *Annals of Otology* **78:** 125–137.

Lim, D. J., Hussl, B. (1969) Human middle ear epithelium. *Archives of Otolaryngology* **89:** 57–71.

Lim, D. J. (1979) Normal and pathological mucosa of the middle ear and eustachian tube. *Clinical Otolaryngology* **4:** 213–234.

Lim, D. J., Shimada, T. (1973) Distribution of mucus-secreting cells in normal middle ear mucosa. *Archives of Otolaryngology* **98:** 2–9.

Proctor, D. F. (1982) Respiratory mucous membrane function. In *The Nose: Upper Airway Physiology and the Atmospheric Environment* (Proctor, D. F., Andersen, I. B., eds.), Elsevier Biomedical Press, Amsterdam, p 131.

Sadé, J. (1967) Ciliary activity and middle ear clearance. *Archives of Otolaryngology* **86:** 128–135.

Shimada, T., Lim, D. J. (1972) Distribution of ciliated cells in the human middle ear. *Annals of Otology* **81:** 203–211.

Tos, M. (1980) Middle ear epithelia in chronic secretory otitis. *Archives of Otolaryngology* **106:** 593–597.

Address for correspondence:
Mr A. M. Agius,
'Serenity',
Bergamott Street,
Mosta MST 07,
Malta.