Scanning Microscopy

Volume 2 | Number 1 Article 51

9-21-1987

Response of Laryngeal and Tracheo-Bronchial Surface Lining to Inhaled Cigarette Smoke in Normal and Vitamin A-Deficient Rats: A Scanning Electron Microscopic Study.

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Scanning Microscopy, Vol. 2, No. 1, 1988 (Pages 545-552)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/88$3.00+.00

RESPONSE OF LARYNGEAL AND TRACHEO-BRONCHIAL SURFACE LINING TO INHALED CIGARETTE SMOKE IN NORMAL AND VITAMIN A-DEFICIENT RATS : A SCANNING ELECTRON MICROSCOPIC STUDY.

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(Received for publication May 06, 1987, and in revised form September 21, 1987)

Abstract

The effects on surface morphology of airway epithelium of cigarette smoke (CS) inhalation alone (experiments one and two) or of CS in combination with hypovitaminosis A (experiment two) was investigated using specific pathogen free rats. Eight morphologically distinct cell types were distinguished overall. Apart from atypical squamous lesions , each of the other cell types could be found in varying proportions in all experimental groups. CS alone caused an increase in the frequency with which intra-lumenal mucus was seen and an increase in the occurrence of secretory cells of types IV (i.e.,'merocrine') and V (i.e.,'apocrine'). In experiment one, the area of trachea covered by cilia as determined by point counting increased significantly ($P<0.01$). Hypovitaminosis A was induced by lowering the dietary intake of vitamin A to a minimum, defined level. Rats showed an approximately 75% decrease in plasma retinol levels and a 95-100% decrease in hepatic stores of vitamin A. At this level, hypovitaminosis A alone had no significant effect on airway epithelial morphology. Foci of squamous metaplasia (squamous cells of type VIIIa) were found in all groups but extensive squamous metaplasia of the larynx and squamous lesions of atypical appearance (type VIIIb) were found only in the vitamin deficient group exposed to CS. The results suggest the synergistic effects of reduced vitamin A and CS may be important in the induction of atypical squamous changes which may predispose the airway to the development of squamous carcinoma.

KEYWORDS: larynx, trachea, bronchi, metaplasia, cigarette smoke, vitamin A-deficiency,scanning electron microscope.

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Introduction

Epidemiology has demonstrated a strong association between cigarette smoking (CS) and lung cancer incidence, which is related both to the duration of smoking and the number of cigarettes smoked (Doll & Hill 1956, Royal College of Physicians Report 1977). Numerous studies have attempted to reproduce this association in experimental animals using chronic inhalation of CS, sometimes for the whole lifespan of the animal (Frasca et al 1968, Kobayashi et al 1974, Smith et al 1978, Davis et al 1975). However, results have not been encouraging, in that there is still no practicable, reproducible animal model of smoke-induced pulmonary cancer.

Smoke inhalation has induced laryngeal tumours in hamsters (Homburger 1975) and at least one study in rats showed an increase from 1% to 9% in the incidence of pulmonary adenomatous tumours (Dalbey et al, 1980). The tumour type contrasts, however, with the predominantly squamous carcinomas found in human smokers. Meade et al (1979) has reported that there is a 50% increase in squamous metaplasia of the larynx in hamsters rendered vitamin A deficient prior to CS exposure. On the basis of the apparent link between vitamin A and carcinogenesis, the aim of the present project was to investigate the effects of cigarette smoke on airway surface morphology either alone or its interaction with hypovitaminosis A. For the scanning electron microscopic component of the study we chose to examine the larynx, trachea and main bronchi, sites at which we expected a relatively heavy deposition of cigarette smoke products and airways which we have previously shown are particularly affected by vitamin A deficiency (Shields & Jeffery 1987).

Materials and Methods

The present SEM study reports the effects of CS in two experiments each forming part of a larger one in which there were light and transmission electron microscopic analyses also. In the first preliminary experiment male Liverpool Hooded rats of average starting weight

300g were used: 4 controls (i.e., 'sham' exposed to air only - Group A) and 4 exposed to cigarette smoke (from 25 cigarettes daily - Group B) for 12 weeks (see Jeffery & Reid 1981; Rogers & Jeffery 1986 for details of exposure conditions).

In the second, male Fischer F344 rats were used and rendered vitamin A-deficient by the following procedure: Weanling male rats (Fischer F344 strain; 50-70 g starting weight) were housed in stainless steel wire cages in laminar flow cabinets, and supplied with filtered water ad libitum. Synthetic diets were manufactured to a recipe described previously (Wise 1982; Shields, Jeffery & Wise, submitted). At the beginning of the experiment, rats were randomly allocated to four treatment groups (Table 1). Only those killed after 12 weeks are considered here.

Table 1. Plan of experiment 2

Group	No of rats n=	Vitamin A content of diet i.u./kg	Exposure
A	7	4000	Sham
B	9	4000	CS
C	7	0-300	Sham
D	9	0-300	CS

Groups A and B received a synthetic diet containing adequate amounts of vitamin A (i.e., 4000 i.u./kg) throughout the experiment. Groups C and D were fed vitamin A-free diet for the first 5 weeks, at which time they were known to be nearing their first weight plateau of vitamin A deficiency (Shields & Jeffery 1987). They were then transferred to a diet low in vitamin A (i.e., 200 i.u./k.g.) but sufficient to maintain their weight.

CS exposure began in the 5th week and lasted for a maximum of 12 weeks. Exposure (Groups B and D) was for 5 days per week, 4 h daily, using a modified Wright's apparatus (Wright 1972). Cigarettes were unfiltered Capstan full strength, 26 mg tar and 2.7 mg nicotine and 16.7 mg carbon monoxide per cigarette (Governmental Chemists' Report 1981) and the animals of experiment two were given smoke from 12 cigarettes daily. Sham-exposed animals (Groups A and C) were placed in identical but smoke-free cabinets for similar 4 h periods. All rats were weighed twice-weekly throughout the experiment.

Rats were killed by i.p. sodium pentobarbitone overdose (Euthatal 120 mg/ml, Hay & Baker Ltd). Plasma and liver were frozen for subsequent vitamin A analysis by fluorimetry (see Shields & Jeffery 1987). Larynx, trachea and main bronchi were removed and fixed by immersion in 3% glutaraldehyde (0.1M cacodylate buffer pH7.2), dehydrated in alcohols to acetone, soaked in liquid CO_2 and critical point-dried. Gold sputter-coated specimens were examined in a Philips 501 B SEM operated at 15kV. A point counting method was used to assess the relative areas covered by cilia in the upper trachea from animals of groups A & B of experiment one. Mean values were determined by calculation of point counts made over mucosa using a 99 point lattice of 1.5 cm spacing overlaying the SEM screen. 11 such grids were placed consecutively along the lateral wall of each trachea. A total of 1089 points were assessed for each specimen at a magnification of 2000 times from each of the 4 animals from each group. Only areas perpendicular to the field of view were taken. Points falling over cracks or contamination were omitted from the count and those falling on the border of two features were 'shared' between the features.

Fig.1. Scanning electron micrograph (SEM) of tracheal surface epithelium of a specific pathogen free rat from group A to show fields of normal cilia (cell type I) interrupted by a focus of squamous metaplasia . Bar = 25 μm.

Fig.2. SEM of apical microvillus border of two tracheal type II "brush" cells (arrows) which contrast with short microvilli of adjacent cells. Group A, Bar = 1 μm.

Fig.3. Central ciliated cell with apical tuft of cilia and intervening long slender microvilli (arrow). The ciliated cell is surrounded by convex apices of type III nonciliated cells each with dense regular array of short microvilli. Rat trachea from group A. Bar = 2.5 μm.

Fig.4. Nonciliated cells, particularly in CS-exposed animals, may also have an apex whose contours show the globular outlines of underlying secretory granules (arrows) (Cell type IV). The plasma membrane immediately over the intracellular granule is usually devoid of microvilli. Group B, Bar = 1 μm.

Fig.5. In contrast with cell type IV, other nonciliated cells (often but not exclusively present in CS exposed rats) had apices bulging into the lumen beyond the ciliary fringe with smooth membranes free of microvilli. Rat trachea from group B,Bar = 10 μm.

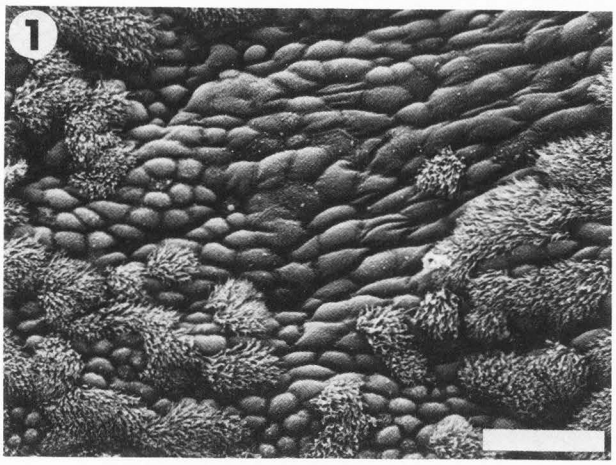
Fig.6. Lateral surface of healthy rat larynx from group A showing a zone transitional to infra-glottal region (left) and vocal fold. The nonciliated cells (type VI) are polygonal and have flat surfaces with few microvilli. Bar = 25 μm.

Results

Cell types

Following examination of all samples in all treatment groups, a number of cell types could be distinguished on the basis of their surface morphologies:

I. Ciliated cells are present in the infra-glottal region of the larynx, trachea and distal bronchi each with cilia of approximately 6 μm in length with intervening microvilli. The continuity of the ciliary fringe is broken by patches or longitudinal tracts of non-ciliated (normal or squamous) cells (Fig.1).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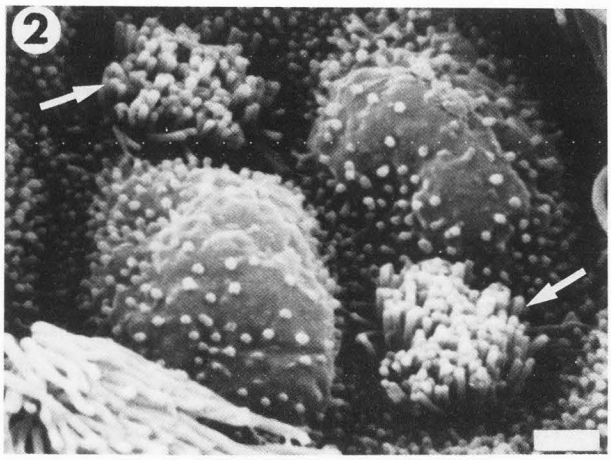
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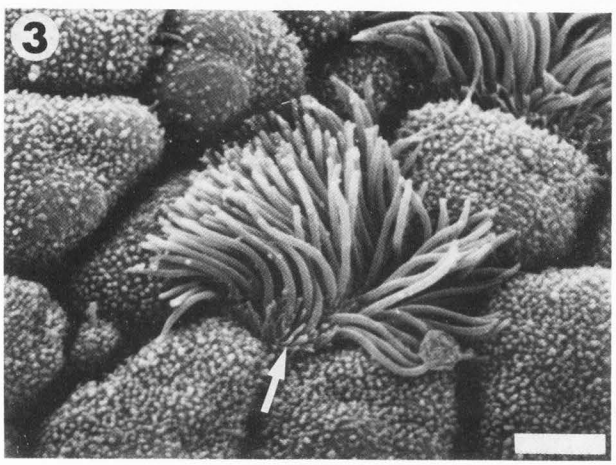
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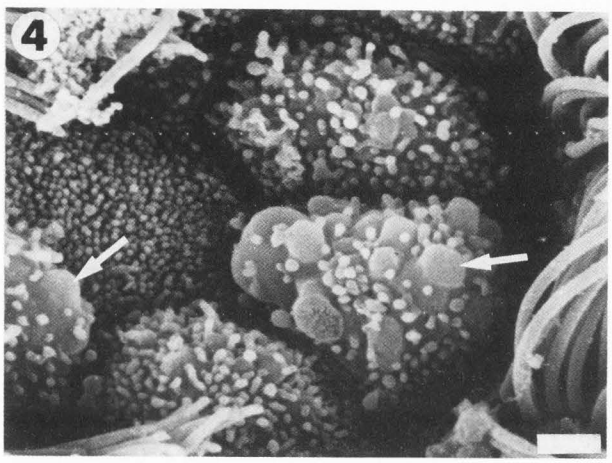
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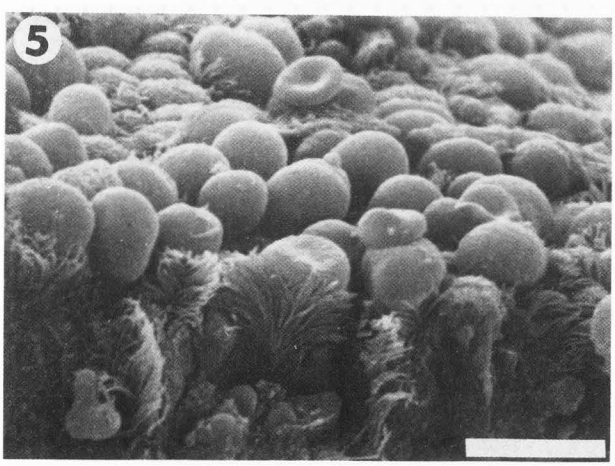
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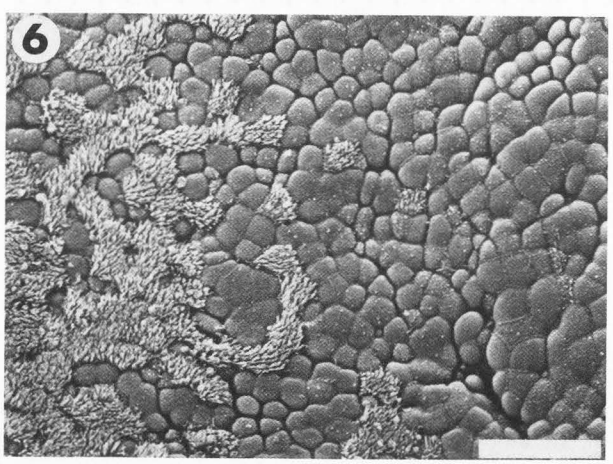
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Nonciliated cells show a variety of forms:
II. "Brush" cells each with a short, relatively thick microvillus brush border (Fig.2).
III. Nonciliated cells normally present in large numbers each with convex apex covered by dense regular array of short microvilli (Fig. 3).
IV. Cells each with bulging apex whose contours show the globular outlines of underlying secretory granules (Fig.4). Whilst the cell surface has microvilli they tend to be sparse or absent from areas overlying secretory granules. Occasionally, isolated granules were found free in the airway lumen (? indicative of merocrine secretion).
V. Cells with apices protruding beyond the ciliary fringe (i.e., greater than 6 μm) whose surfaces were free of microvillus covering (Fig. 5) (? indicative of apocrine secretion).
VI. Cells with flat, polygonal surfaces of about 10um maximum diameter with sparse microvilli (Fig.6).
VII. Squamous cells of the laryngeal vocal fold each about 30 μm in diameter with ruffled or micro-ridged surface (Fig.7).
VIII. a) Isolated foci of squamous metaplastic cells, angular or spindle-shaped with smooth surface (see Fig 1) or b) squamous cells in the form of atypical foci: i.e., elongate or spindle-shaped and usually without microvilli. Each usually appears raised and thrown into folds or the cells may form a concentric ring with their long axes radiating from a central depression in the mucosa (Fig.8).

In addition desquamated epithelial cells may be found lying free in the airway lumen, as are microvillus covered 'bodies', (? macrophages). Random fractures or cuts across the mucosal wall of the upper trachea and larynx exposed the internal microstructure of submucosal glands and their ducts (Figs.9 & 10).

Normal appearance and effect of CS

Two to three undulations traverse the laryngeal wall as its lumen widens caudal to the vocal fold. The squamous epithelium (cell type VII) of the vocal fold gives way to a non-ciliated one with smaller, flat angular cells (type VI) which form a transitional zone before patches of ciliated cells begin. Ciliated (type I) and nonciliated (type III) cells is then the pattern characteristic of the surface of the trachea and main bronchi. Isolated foci of squamous cells may be found in control (healthy) animals, particularly in the zone immediately caudal to the first transverse undulation of the larynx. The area covered by cilia constitutes about 56% of the entire surface of the upper 1/3rd of the trachea (see table 2). However the division of the main carina (trachea into main bronchi) is invariably nonciliated (type III) or squamous (type VII). The mucosa of the main bronchus is thrown into longitudinal folds covered by both ciliated and nonciliated (type III) cells but the latter are occasionally secretory (type V). Mucus (remaining after fixation and processing) may be seen but rarely. Free microvillus-covered bodies of 10-20 μm diameter (possibly macrophages) are infrequently present.

Fig.7. Normal squamous cells of the laryngeal vocal fold each of approximately 30 μm max. diameter, angular in shape, often 'flaking' (particularly in vitamin A deficiency) with a surface showing ruffles or "microridges". Cell outlines (arrows). Group A, Bar = 5 μm.

Fig.8. Squamous cell lesions of atypical appearance in that they were only found in the vitamin A deficient group given CS (Group D). The raised foci consisted of elongate, spindle shaped cells usually forming a concentric ring with their long axes radiating from a central depression in the mucosa(arrow). Rat larynx. Bar = 50 μm.

Fig.9. Opening of ciliated submucosal gland duct onto surface of upper trachea. Group A, Bar = 10 μm.

Fig.10. Chance fracture of laryngeal/tracheal submucosal gland showing secretory (s) and ductal (d) regions. A bronchial vessel (arrow) is seen between gland and surface epithelium which is uppermost. Group B, Bar = 25 μm.

Table 2. Mean % values for tracheal ciliated (CC) and nonciliated (NC) cells (± SEM): Experiment 1.

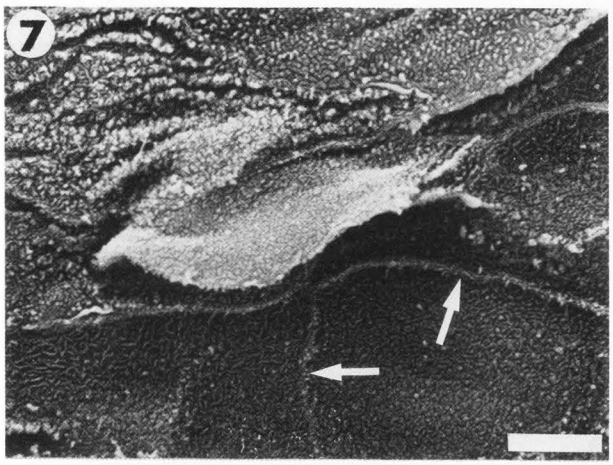
Group	CC	NC
A (control)	56.3 (1.8)	43.7 (1.8)
B (CS alone)	65.7* (3.1)	34.0* (3.1)

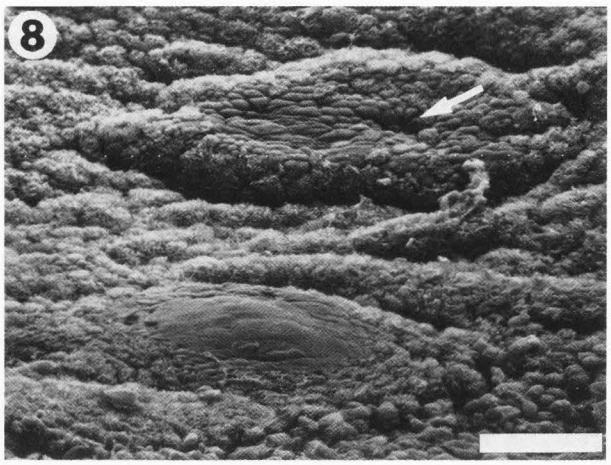
*Significantly different from control mean ($P<0.01$).

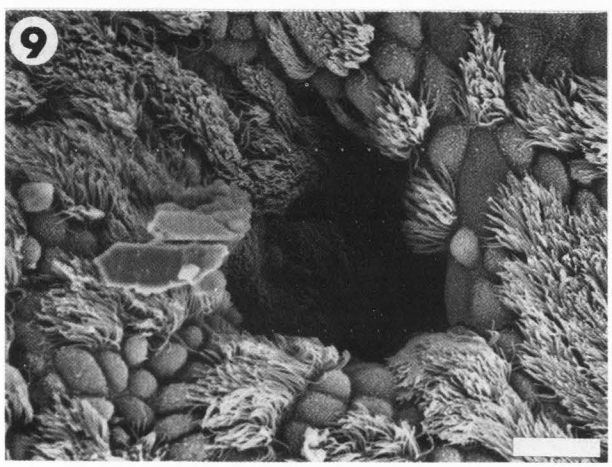
Sub-acute exposure to cigarette smoke did not cause dramatic changes in surface morphology. One animal showed two extensive areas of squamous metaplasia (cell type VII). Mucus as sheets or flakes was found associated with the main carina (which was invariably squamous) and was also found in the main bronchus (Fig.11). Secretory cells of type V were found in CS-exposed animals of experiment one and predominated in 4 of the 9 animals exposed to CS in experiment two. Pre-ciliated or brush cells (type II) were apparent and secretory cells of type IV were often found. The extent to which the surface of the trachea was covered by cilia was assessed (table 2). Exposure to CS caused a significant ($P<0.01$) increase in coverage by cilia when compared with respective controls.

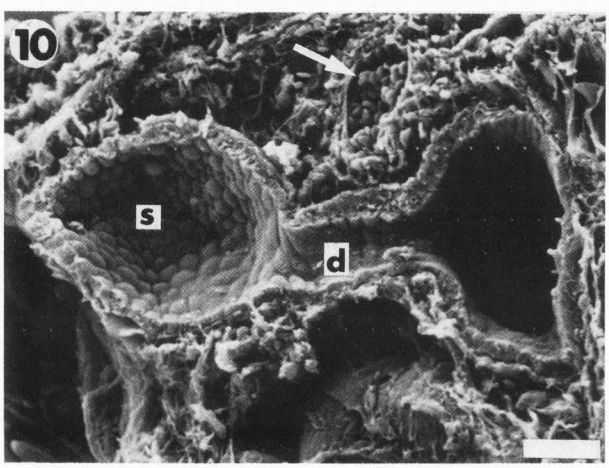
Effect of vitamin A-deficiency with or without CS

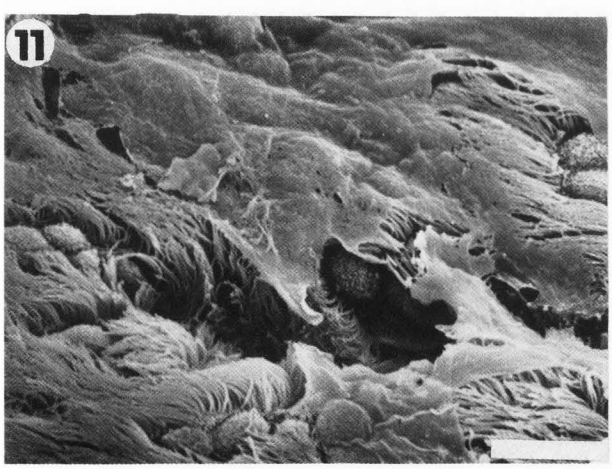
There were few changes in animals rendered vitamin A deficient alone. Cilia were present in most regions but for isolated small patches











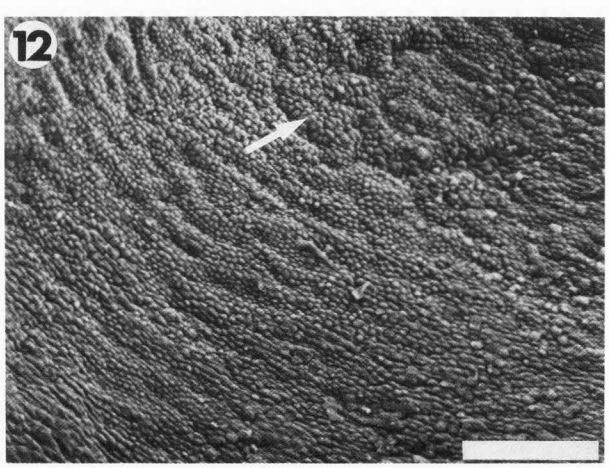


Fig.11. Mucus present as a sheet overlying the ciliated epithelium of the main bronchus. Group A, Bar = 10 μm.

Fig.12. Complete squamous metaplasia of the infra-glottal region of the larynx from a vitamin A deficient animal also given cigarette smoke for 2 weeks. The surface epithelium is comprised of squamous cells (type VIII a) frequently organised into 'atypical' foci (VIIIb, arrow). Group D, Bar = 100 μm.

of squamous metaplasia in the larynges of 3 animals. The 'transitional' zone of non-ciliated cells (type VI) was lost in the larynx and ciliated cells appeared to extend in a rostral direction onto the edge of the vocal fold. The vocal fold itself remained squamous but many more cells appeared to be flaking from the surface than seen in healthy controls (see Fig 7). Macrophage-like cells were frequently found in the larynx, trachea and main bronchi. There was little mucus.

In contrast, exposure of the vitamin A-deficient animals to CS resulted in widespread squamous metaplasia. Every animal except one (which was heavily ciliated) had extensive areas of squamous epithelium (cell type VIII) comprising 50% of the entire surface in 3 or nearly complete in 5 (Fig 12). Mucus was present in excess of that seen in the other three experimental groups, secretory cells were usually of the apocrine type (see Fig 5) and cilia often appeared entangled in mucus. Two of the 9 animals showed several raised lesions in their laryngeal epithelia. Each lesion was squamous, on one occasion of type VIIIa but otherwise of type VIIIb squamous cells (see Figs 8 & 12). The latter were never found in untreated or vitamin A-deficient controls and in this sense were atypical. The extent of these changes bore no correlation with the individual levels of circulating plasma or hepatic stores of vitamin A.

Table 3. Cell types by surface morphology - Summary

I	Ciliated
II	Pre-ciliated or brush
III	NCC convex ($\sim$10μm), microvillus
IV	NCC-secretory convex - globules
V	NCC (? secretory) - bulging - smooth
VI	NCC - flat - polygonal ($\sim$ 10 μm) - sparse microvilli
VII	Squamous - flat - angular ($\sim$ 30μm) - microridges
VIIIa.	Squamous - spindle/elongate - variable form - few microvilli
b.	Raised lesions often radially arranged

Discussion

Cell types

The variety of cell types present in the surface epithelium of rat airway and their ultrastructure has been reviewed (Jeffery & Reid 1975, Jeffery 1983) and ultrastructural changes due to inhalation of cigarette smoke have been reported (Jeffery & Reid 1981). The present study describes cell 'types' discriminated on the basis of surface morphology (summarized in Table 3). The correlation of cell types by SEM with those seen by TEM is possible: ciliated cells are easily identified by both methods of examination, type II cells by SEM probably correspond with the brush cells identified in the rat, type III cells with serous cells or those of indeterminate morphology (previously referred to as "intermediate" cells) and type IV with the goblet or mucous cell distended by mucous granules (Jeffery & Reid 1975, Jeffery 1983). Those of type V probably represent the ballooning of cell apices due to confluence of underlying mucous and eventual apocrine secretion. They may also indicate damage to a variety of cell types due to cell toxicity: the exact nature of the protrusions requires further investigation. The TEM ultrastructure of the type VI cells seen by SEM is unknown and our ongoing studies are aimed at characterization of this cell type and also the squamous cells (types VII & VIII) found by SEM.

Cigarette smoke

Two of the CS-induced changes seen by SEM corroborate light microscopic and TEM findings. The greater frequency of cell type IV corresponds with the mucous cell hyperplasia previously reported (Jones et al 1972, Jeffery & Reid 1981, Rogers & Jeffery 1986). The increase in surface area covered by cilia is also in accord with the TEM study reported previously (Jeffery & Reid 1981) in which 2 or 6 weeks exposure to CS induces an increase in the number of ciliated cells each of which retains cilia of normal structure and concentration. It is the authors' opinion that whilst the present experiments are of relatively short duration in relation to the life-span of the animal they do indicate that factors other than CS (e.g., infection) are important determinants of ciliary damage associated with the post-mortem findings in patients dying of smoking-related disease (Chang 1957).

Vitamin A deficiency and CS

Squamous metaplasia is associated with, and possibly predisposes to, the development of carcinoma *in situ* and invasive squamous carcinoma (McDowell & Trump 1984, Spencer 1985). Since the majority of lung tumours are squamous we have explored the effects of combining the effects of both vitamin A deficiency and CS, the former implicated by epidemiological studies (Bjelke 1975, Wald et al 1980, Kark et al 1981, Shekelle et al 1981) and the latter a recognized agent in the causation of lung cancer (Doll & Hill 1956, Royal College of Physicians Report 1977). When animals are rendered vitamin A-deficient alone, squamous metaplasia is patchy and restricted to extrapulmonary airways as described previously (Wolbach & Howe 1925, Wong & Buck 1971, Anzano et al 1980, Shields & Jeffery 1987). However in combination with CS there is a dramatic change in surface morphology from ciliated to squamous with several atypical foci. The changes are most obvious in the infra-glottal region of the larynx and support the earlier findings of Meade et al (1979) where there was a 50% increase in the incidence of CS-induced (6 weeks exposure) squamous metaplasia of the larynx. The results support the view that vitamin A deficiency encourages the early development of epidermoid metaplasia in response to inhaled CS and shows that 'atypical' lesions develop in the larynx within a relatively short time span. The rat model is amenable to further experimentation and manipulation as a means of investigating squamous metaplasia and early changes in carcinogenesis.

Acknowledgements

We are grateful to the Cancer Research Campaign (UK) for supporting PAS and PKJ in this work, Miss Fiona Nelson for her technical help and Mrs Jenny Billingham for her patience and accuracy in typing the manuscript.

References

1. Anzano MA, Olson JA, Lamb AJ. (1980). Morphologic alterations in the trachea and the salivary gland following the induction of rapid synchronous vitamin A deficiency in rats. Am J Path. 98:717-732.

2. Bjelke E. (1975). Dietary vitamin A and human lung cancer. Int J Cancer 15, 561-565.

3. Chang SC. (1957). Microscopic properties of whole mounts and sections of human bronchial epithelium of smokers and non-smokers. Cancer 10, 1246.

4. Dalbey WE, Nettesheim P, Griesemer R, Caton JE, Guerin MR. (1980). Chronic inhalation of cigarette smoke by F344 rats. J. Nat. Cancer Inst. 64, 383.

5. Davis BR, Whitehead JK, Gill ME, Lee PN, Butterworth AD, Roe FJC. (1975). Response of rat lung to inhaled tobacco smoke with or without prior exposure to 3, 4-benzpyrene (BP) given by intra-tracheal instillation. Br J Cancer 31, 469-484.

6. Doll R, Hill AB. (1956). Lung cancer and other causes of death in relation to smoking: a second report on mortality of British doctors. Brit Med J. ii, 1071-1081.

7. Frasca JM, Auerbach O, Parks VR, Jamieson JD. (1968). Electron microscopic observation of the bronchial epithelium of dogs: II Smoking Dogs. Exp Molec Path. 9, 380-399.

8. Homburger F. (1975). Smoker's larynx and carcinoma of the larynx in Syrian hamsters exposed to cigarette smoke. Laryngoscope 85, 1874-1881.

9. Jeffery PK. (1983). Aetiology and pathogenesis of chronic air flow limitation. Resp. Dis. Pract. 1, 4-6.

10. Jeffery PK, Reid L. (1975). New observations of rat airway epithelium: a quantitative and electron microscopic study. J Anat. 120, 295.

11. Jeffery PK, Reid L. (1981). The effect of tobacco smoke with or without phenylmethyl-oxadiazole (PMO) on rat bronchial epithelium: a light and electron microscopic study. J Path. 133, 231-359.

12. Jones R, Bolduc P, Reid LM. (1972). Protection of rat bronchial epithelium against tobacco smoke. Brit Med J. 2, 142-144.

13. Kark JD, Smith AH, Switzer BR, Hames CG. (1981). Serum vitamin A (retinol) and cancer incidence in Evans County, Georgia. J. Nat. Cancer Inst. 66, 7-16.

14. Kobayashi N, Hoffman D, Wynder EL (1974). A study of tobacco carcinogenesis XII. Epithelial changes induced in the upper respiratory tracts of Syrian golden hamsters by cigarette smoke. J. Nat. Cancer Inst. 53, 1085-1089.

15. McDowell EM, Trump BF. (1984). Histogenesis of preneoplastic and neoplastic lesions in tracheobronchial epithelium. Surv. Synth. Path. Res. 2, 235-279.

16. Meade PD, Yamashiro S, Harada T, Basrur PK. (1979). Influence of vitamin A on the laryngeal response of hamsters exposed to cigarette smoke. Prog. Exp. Tumor Res. 24, 320-329.

17. Rogers DF, Jeffery PK. (1986) Inhibition by oral N-acetylcysteine of cigarette smoke-induced "bronchitis" in the rat. Exp. Lung Res. 10, 267-283.

18. Royal College of Physicians' Report (1977). Smoking or Health. Pub. Pitman Med., London.

19. Shekelle RB, Lepper M, Liu S, Maliza C, Raynor WJ, Ropssof AH, Paul O, Shryock AM, Stanley J. (1981). Dietary vitamin A and the risk of cancer in the Western Electric study. Lancet ii, 1185-1189.

20. Shields PA, Jeffery PK. (1987) The effects of inhalation of cigarette smoke on airway epithelial morphology in vitamin A-deficient F344 rats. I Two weeks' exposure. Brit. J. Exp. Path. 68, (in press).

21. Smith G, Wilton LV, Binns R. (1978). Sequential changes in the structure of the rat respiratory system during and after exposure to tobacco smoke. Toxicol. Appl. Pharmacol. 46, 579-591.

22. Spencer H. (1985) Pathology of the Lung Vol 1., 4th ed. Pergamon Press. Oxford pp.851-866.

23. Wald N, Idle M, Boreham J, Bailey A. (1980). Low serum vitamin A and subsequent risk of cancer. Lancet ii, 813-815.

24. Wise A. (1982). Interaction of diet and toxicity - the future role of purified diet in toxicological research. Arch. Toxicol., 50, 287-299.

25. Wolbach SB, Howe PR. (1925). Tissue changes following deprivation of fat-soluble A vitamin. J. Exp. Med. 42, 753-777.

26. Wong Y, Buck RC. (1971). An electron microscopic study of metaplasia of the rat tracheal epithelium in vitamin A deficiency. Lab. Invest. 24, 55-56.

27. Wright BM. (1972). Cigarette smoking for animal experiments. Lab. Pract., 21, 881.

Discussion with Reviewers

M. Albertsson: How was the dose of CS exposure selected?

Authors: The dose was selected from the results of previous experiments in which the extent of mucous (i.e., goblet) cell hyperplasia was the end point assessed. Carboxyhaemoglobin levels were of the order of 15%, similar to that of a heavy smoker.

B. Afzelius: The area covered by ciliated cells in the upper part of the rat trachea is given as 56%. Do you have a figure also for the lower part of the trachea and for the bronchus?

Authors: We have not analysed quantitatively the area covered by cilia in the lower trachea and main bronchus. In our previous studies by transmission microscopy (Jeffery & Reid, 1975, text ref) we found the % of ciliated cells in the upper and lower trachea and main bronchus to be 49, 85 and 79, respectively. The percentage increased further in intrapulmonary airways.

B. Afzelius: Vitamin A deficiency in combination with smoking evidently provides a risk of squamous metaplasia (and probably of squamous carcinoma). Do you believe that vitamin A excess on the other hand would give some protection to these conditions?

Authors: Yes, there is evidence that high concentrations of vitamin A can reverse metaplasic changes in both animals and man (Clamon et al. (1974). Alpha- and beta-retinyl acetate reverse metaplasias of vitamin A deficiency in hamster trachea in organ culture. Nature 250, 64-66; Gouveia J et al. (1982) Degree of bronchial metaplasia in heavy smokers and its regression after treatment with a retinoid. Lancet i:710-712) but in a prophylactic regimen its toxicity would require serious consideration.