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The effect of airway epithelium on smooth muscle contractility in bovine trachea

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1 In bovine tracheal smooth muscle the presence of airway epithelium significantly reduced the sensitivity and maximum contractile response to histamine, 5-hydroxytryptamine (5-HT) or acetylcholine.

2 Muscle contraction induced by K^+ and electrical field stimulation was of similar magnitude both in the presence or absence of adherent epithelium.

3 The effect of epithelium on smooth muscle contractility was unaffected by pretreatment with indomethacin (10^{-6}M) or mepacrine (5×10^{-5}M).

4 The relaxant response to isoprenaline was enhanced in the presence of epithelium, although this was significant only in the case of precontraction with 5-HT.

5 It is concluded that the bronchial epithelium may produce a relaxant factor which is not a cyclooxygenase or lipoxygenase product. The production of this factor may be reduced or lost following epithelial damage and this may be important in the pathogenesis of bronchial hyperresponsiveness in asthma.

Introduction

The bronchial epithelium of asthmatics is abnormal both in fatal attacks (Dunnill, 1960; Cutz *et al.*, 1978) and during remissions (Laitinen *et al.*, 1985). During exacerbations of extrinsic asthma there is infiltration of the bronchial wall with eosinophils and release of eosinophilic products such as major basic protein (MBP) which may cause airway epithelial damage (Frigas *et al.*, 1981; Filley *et al.*, 1982). Infection with respiratory viruses causes epithelial shedding (Hers, 1966) and makes some normal subjects hyperreactive (Empey *et al.*, 1976). Airway inflammation and epithelial damage is a prominent feature of both mild and severe asthma and may have a role in bronchial responsiveness (Laitinen *et al.*, 1985; Boushey & Holtzman, 1985). In preliminary studies it has been suggested that removal of epithelium from canine airways increases the sensitivity of the bronchial smooth muscle to acetylcholine and 5-hydroxytryptamine (5-HT) (Aarrhus *et al.*, 1984). We have further characterized how airway epithelium may modulate the contractility of airway tracheal smooth muscle in bovine trachea, in which the epithelium is easy to remove mechanically.

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Methods

Tissue preparation

Tracheae were removed from young cattle (< 2 years) within 5–10 min of slaughter and exsanguination, placed in Krebs solution pre-gassed with 5% CO_2 in O_2 at 4°C, and returned to the laboratory within 60 min. Strips of smooth muscle, 1 cm long, with adherent epithelium were cut from the trachea and tied at each end with silk thread. Epithelium and submucosa were carefully removed from half the strips leaving smooth muscle only (Figure 1). Both preparations were mounted for isometric recording in 10 ml tissue baths at 37°C containing Krebs solution continuously gassed with 5% CO_2 in O_2.

Muscle responses

Tension was measured with Grass FT.03 transducers (Grass Instruments Co., Quincy, Mass., U.S.A.) and responses recorded on a Grass 7D polygraph. A tension of 5 g, which in preliminary length-tension studies was found to be optimal, was applied to the strips and they were allowed to equilibrate for 90 min

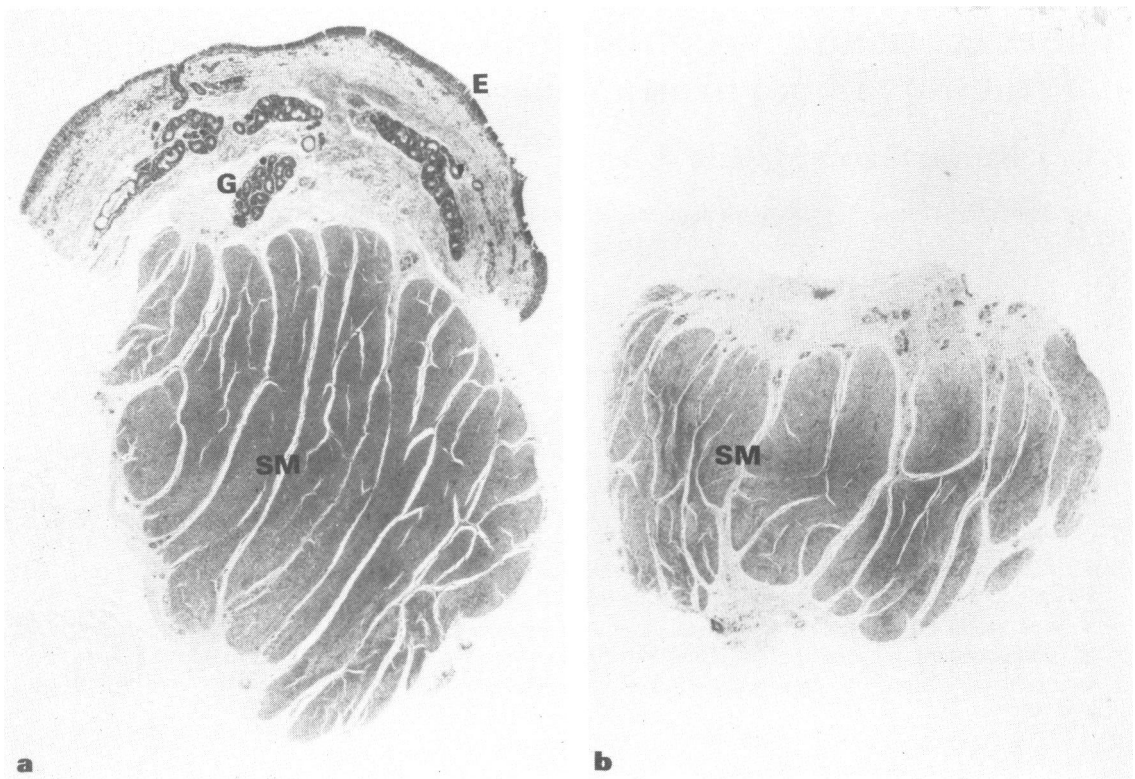

Figure 1 Photomicrographs of tranverse sections of bovine trachea stained with haematoxylin and eosin, showing in (a) the epithelium (EP), submucosal glands (G) and smooth muscle (SM) and in (b) smooth muscle after epithelial removal. Magnification × 40.

during which time they were washed four times. Cumulative concentration-response curves were constructed for acetylcholine (ACh), histamine, 5-HT and potassium (K^+) and frequency-response curves to electrical field stimulation. In further experiments, the tension of the muscle strips was increased by addition of the concentration of ACh, histamine, or 5-HT giving about 80% of their respective maximal contraction (EC_{80}). For each spasmogen, concentration-response curves to isoprenaline were then obtained. At the end of each experiment, the epithelium, if present, was removed and each muscle was weighed, the contractions being corrected for the weight of muscle to allow direct comparison of responses.

The effects of possible inhibitors of an epithelial factor were also assessed. Acetylcholine concentra-

Table 1 Contractile responses in bovine tracheal smooth muscle

	Maximal contraction with epithelium present†	*EC_{50} (M) Epithelium*		
		Absent	*Present*	P
Histamine	*53 ± 12	**5.7×10^{-6}	1.2×10^{-5}	<0.05
Acetylcholine	71 ± 8	5.0×10^{-6}	2.5×10^{-5}	<0.05
5-Hydroxytryptamine	60 ± 14	8.0×10^{-7}	1.7×10^{-6}	<0.05
K^+	97 ± 16	3.0×10^{-2}	3.0×10^{-2}	NS

† Expressed as % of maximal contraction elicited by each agonist on tissues without epithelium.
Results show * arithmetic means (± s.e.means) and ** geometric means from 10 specimens.

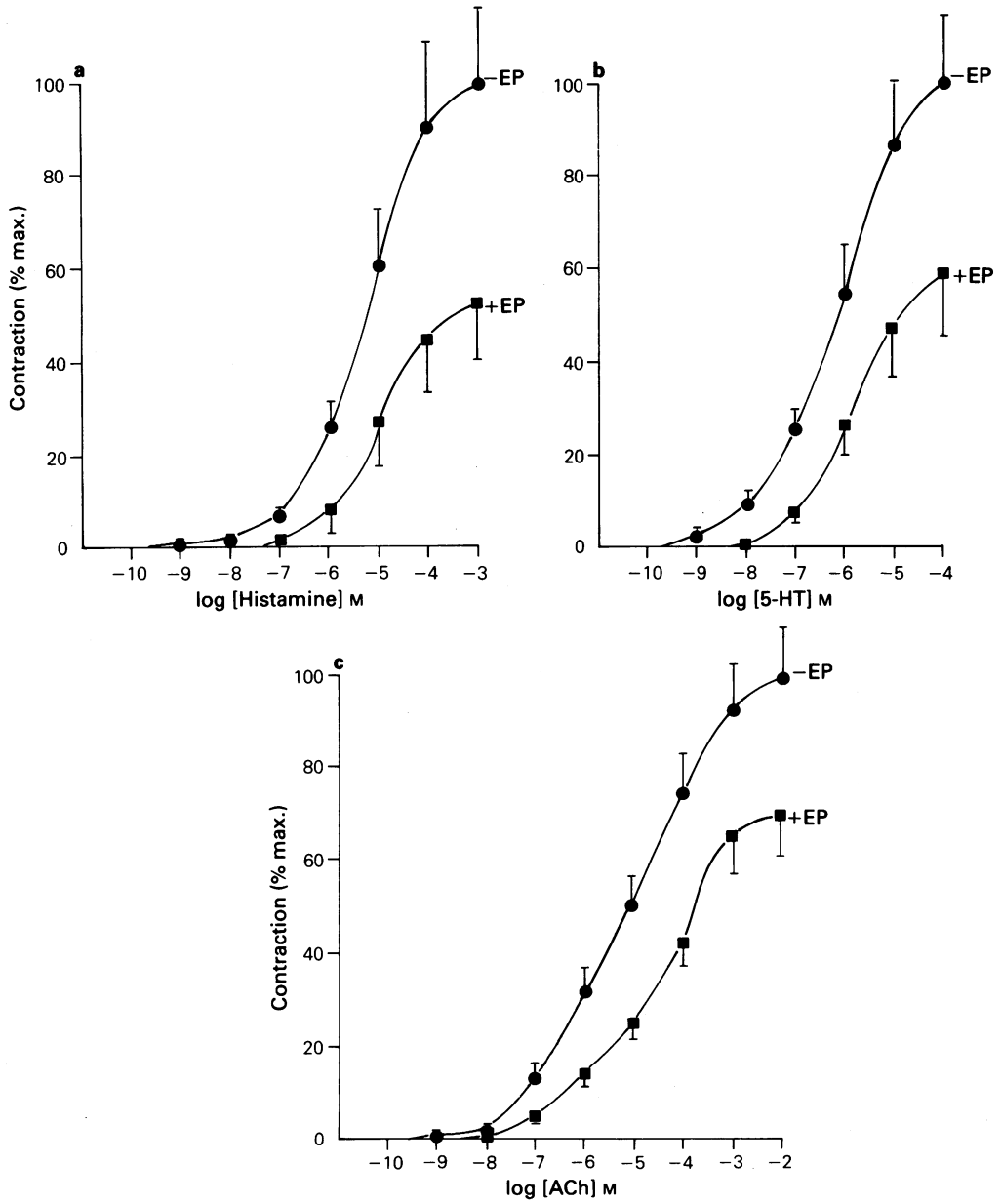

Figure 2 Responses of bovine airway smooth muscle, with (+ EP) and without (− EP) adherent epithelium, to (a) histamine, (b) 5-hydroxytryptamine (5-HT) and (c) acetylcholine (ACh). The abscissa scales indicate the concentration of agonist (M) on a logarithmic scale. The ordinate scales represent the response as a % of the maximal response without epithelium, corrected for the weight of muscle strips: (■) with and (●) without epithelium. Points represent the mean, and vertical lines show s.e.mean, from 10 observations.

tion-response curves were constructed and then the strips were washed until the tension returned to baseline. The muscle strips were then treated with indomethacin (10^{-6}M) or mepacrine (5×10^{-5}M) for 30 min and concentration-response curves to ACh repeated.

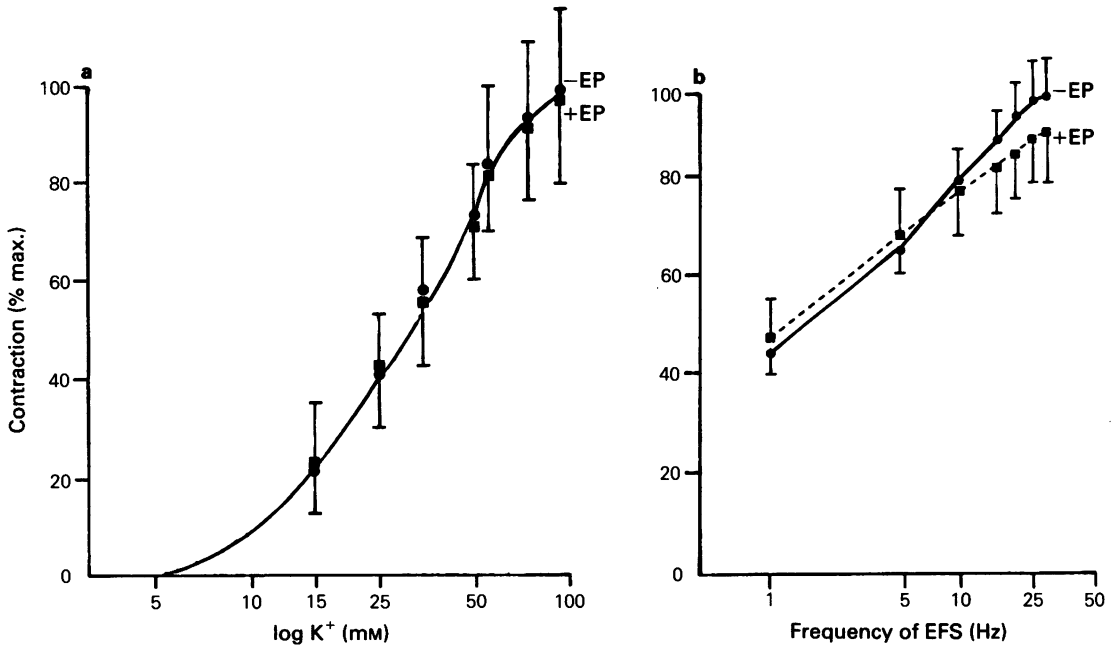

Figure 3 Responses of bovine airway smooth muscle, with (+ EP) and without (− EP) adherent epithelium, to (a) potassium (K^+) and (b) electrical field stimulation (EFS). The abscissa scales indicate (a) the concentration of K^+ (mM) and (b) the frequency of EFS (Hz) on logarithmic scales. The ordinate scales represent the response as a % of the maximal response without epithelium, corrected for the weight of muscle strips: (■) with and (●) without epithelium. Points represent the mean, and vertical lines show s.e.mean, from 10 observations.

Electrical field stimulation

Electrical field stimulation was generated by a Grass S4 stimulator (Grass Instrument Co, Quincy, Mass., U.S.A.) via parallel platinum wire electrodes. Stimulation consisted of 20 s of 50V biphasic, square wave pulses of 1 ms duration with frequencies varying between 1 and 30 Hz. Maximal cholinoceptor mediated responses occurred at 25–30 Hz and could be abolished by atropine (10^{-5}M) or tetrodotoxin (3×10^{-6}M). A separate amplifier was connected to the output of the stimulator to maintain constant current during stimulation (Pantechnic, Liverpool).

Table 2 Relaxation responses to isoprenaline in precontracted bovine tracheal smooth muscle

Precontraction agent	EC_{50} (M) *Epithelium* *Absent*	*Present*	P
Histamine	7.0×10^{-8}	5.9×10^{-8}	NS
Acetylcholine	3.4×10^{-6}	2.3×10^{-6}	NS
5-Hydroxytryptamine	2.1×10^{-7}	1.1×10^{-7}	<0.05

Data shown are geometric means from 10 specimens.

Drugs and solutions

The Krebs solution was of the following composition (mM): NaCl 118, KCl 5.9, $MgSO_4 7H_2O$ 1.2, $CaCl_2 6H_2O$ 2.5, $NaH_2PO_4 H_2O$ 1.2, $NaHCO_3$ 26 and glucose 11. The responses to different concentrations of K^+ included the concentration of K^+ present in the Krebs solution.

The following drugs were used: acetylcholine chloride, histamine dichloride, 5-hydroxytryptamine creatinine sulphate complex, isoprenaline hydrochloride, indomethacin and mepacrine (Sigma). The histamine dichloride, 5-HT, acetylcholine and mepacrine were made up in deionised water daily. The isoprenaline was made up with ascorbate (0.1 mg ml^{-1}) as a stock solution and stored at − 20°C. The indomethacin was dissolved in sodium bicarbonate solution (100 mM) and kept at − 20°C.

Statistical analysis of results

Differences between the log EC_{50} and the maximal corrected contractions of the individual preparations were analysed by an unpaired Student's *t* test.

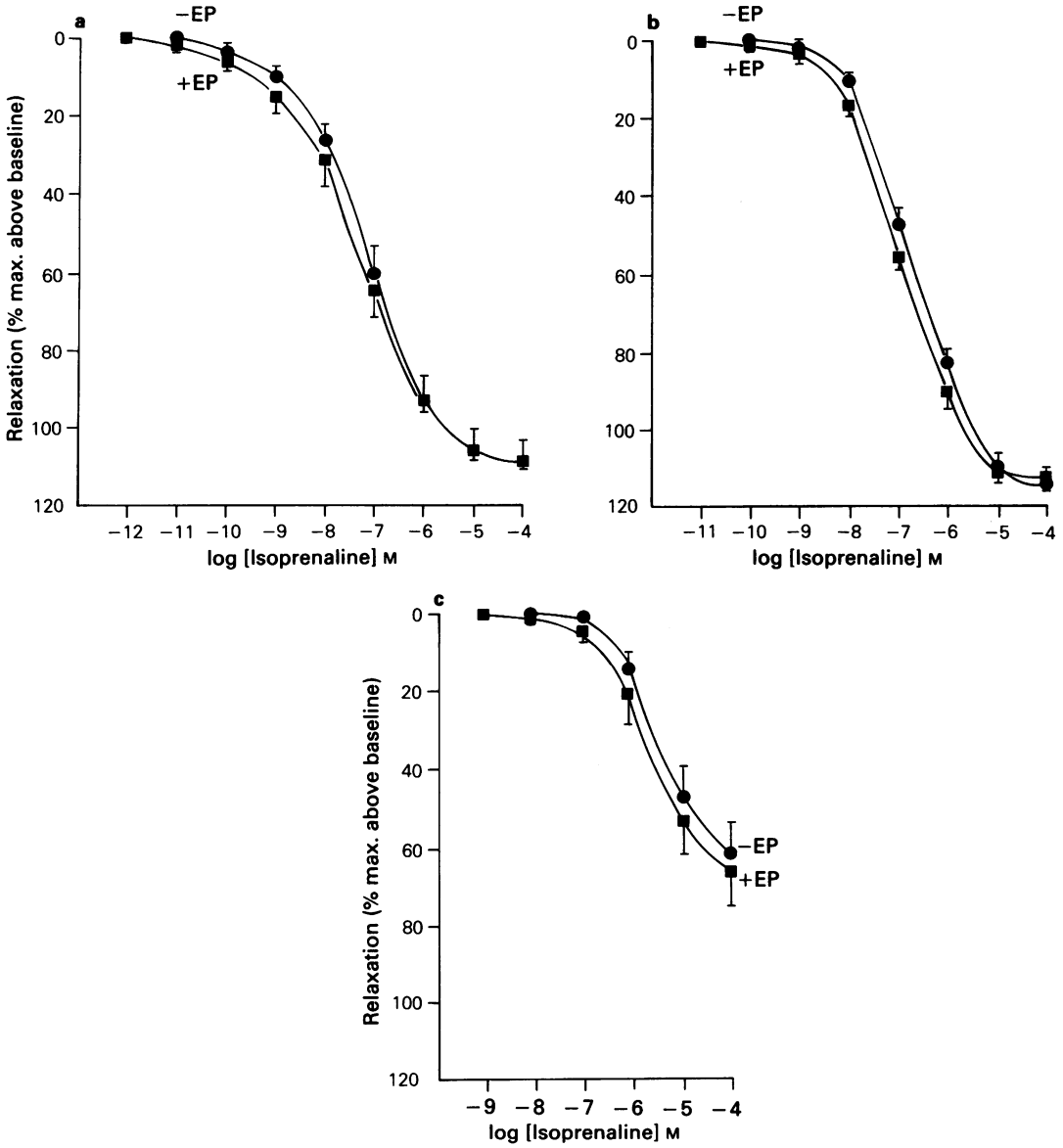

Figure 4 Responses of bovine airway smooth muscle, with (+ EP) and without (− EP) adherent epithelium, to isoprenaline following precontraction with the concentrations of (a) histamine, (b) 5-hydroxytryptamine and (c) acetylcholine equivalent to their EC_{80} values. The abscissa scales indicate the concentration of isoprenaline (M) on a logarithmic scale. The ordinate scales represent the relaxation response as a % of the maximal contraction: (■) with and (●) without epithelium. Points represent the mean, and vertical lines show s.e.mean, from 10 observations.

Results

Effect of epithelium on contraction

Muscle strips without epithelium were more sensitive to histamine, 5-HT and ACh both in terms of maximal effect and EC_{50} (Figure 2; Table 1). However, there was no difference in the K^+- and electrical field stimulation-induced contraction between the two preparations (Figure 3). Neither the EC_{50} nor the maximal response to ACh was significantly changed by indomethacin (10^{-6}M) or mepacrine (5×10^{-5}M).

Effect of epithelium on relaxation

Isoprenaline was more effective at relaxing the smooth muscle with adherent epithelium (Figure 4), although this difference was significant only in preparations precontracted with 5-HT (Table 2). Isoprenaline was more effective at relaxing airways precontracted with 5-HT and histamine than those contracted with ACh.

Discussion

We have demonstrated that removal of the epithelium from bovine tracheal smooth muscle significantly increases its sensitivity to ACh, 5-HT and histamine, but not to K^+ or electrical field stimulation. Isoprenaline is slightly more potent when the epithelium is present. Muscle contractility is unchanged by the presence or absence of epithelium because the response to K^+ or electrical field stimulation is similar in the two preparations. We consider it unlikely that epithelium acts as a diffusion barrier to reduce access of spasmogens because the majority of the muscle surface is exposed in both preparations and the relaxation responses to isoprenaline were not reduced in the presence of epithelium. The reduction in responses to ACh, histamine and 5-HT in the presence of epithelium could be explained by the release of an epithelial factor which causes smooth muscle relaxation. This may be analagous to the factor produced by vascular endothelium which elicits vascular smooth muscle relaxation *in vitro* (Furchgott & Zawadski, 1980; Furchgott, 1984; Griffith *et al.*, 1984). Endothelium derived relaxant factor (EDRF) production can be prevented physically by damaging the endothelium or its effect blocked by mepacrine (1×10^{-5}–3×10^{-5}M), a phospolipase A_2 inhibitor. We were unable to block the airway epithelial effect with either mepacrine or indomethacin, which implies that this putative factor is not a lipoxygenase or cyclo-oxygenase product. The similar response of each preparation following K^+ may be explained by direct depolarization of the muscle or possibly by damage to the epithelium by increasing concentrations of K^+.

Non-asthmatics are less sensitive than asthmatics to various inhaled stimuli such as histamine, cold air or ozone and they reach a point where larger doses of histamine or methacholine cause no further bronchoconstriction (Woolcock *et al.*, 1984; Sterk *et al.*, 1985). Asthmatics continue to bronchoconstrict in a dose-related fashion and seem to lack a 'braking mechanism' to prevent excessive degrees of bronchoconstriction. A relaxant factor generated by the bronchial epithelium could act as a 'braking mechanism', but its production, like that of EDRF, might be reduced by cellular damage. A prominent histological feature of lung specimens obtained *portmortem*, from asthmatics dying of acute asthma is damage and shedding of the bronchial epithelium (Dunnill, 1960; Cutz *et al.*, 1978). Epithelial cells appear in the sputum of asthmatics during exacerbations, presumably as a result of their loss from the airways (Naylor, 1962; Sanerkin & Evans, 1965). Bronchial biopsies, obtained from mild and severe asthmatics in remission also show changes in epithelial structure, not present in biopsies from normal subjects (Laitinen *et al.*, 1985). These epithelial abnormalities are evident from both light and electron microscopic examination throughout the tissue.

There is increasing interest in the interaction of epithelial and inflammatory cells in models of asthma (Boushey & Holtzman, 1985) and *in vitro* epithelial cells can elaborate lipoxygenase metabolites from arachidonic acid (Holtzman *et al.*, 1983). Airway inflammation occurs in animal models of hyperreactivity and acute asthma in man. For example administration of ozone to guinea-pigs produces damage to the epithelium and increases in bronchial responsiveness followed by migration of inflammatory cells into the bronchial wall (Murlas & Roum, 1985). In exacerbations of extrinsic asthma, eosinophils accumulate in the bronchial submucosa and release of their products, such as major basic protein (MBP) occurs. MBP damages both animal and human airway epithelium *in vitro* (Frigas *et al.*, 1980; 1981), is present in close proximity to areas of epithelial damage in *postmortem* specimens from patients who died from asthma and is found in expectorated sputum and mucus plugs during exacerbations of asthma (Filley *et al.*, 1982). Viral infections of the respiratory tract may lead to shedding of the bronchial epithelium (Hers, 1966) and an increase in bronchial hyperreactivity has been described in some non-asthmatic subjects after infection with respiratory viruses (Empey *et al.*, 1976). Bronchial reactivity may also increase after administration of live attenuated viruses but only if infection has occurred, as demonstrated by a rise in viral titres (Laitinen *et al.*, 1976).

There is evidence that asthma is associated with airways inflammation and epithelial damage and that increases in bronchial responsiveness can occur after infection with respiratory viruses which cause shedding of epithelium. MBP and other inflammatory products, like the more potent eosinophilic cationic protein, may, by damaging epithelium, also be responsible for increasing airway responsiveness. Cultured airway epithelium cells are able to produce lipoxygenase metabolites so they may also elaborate a factor with a similar effect on bronchial smooth muscle as that of EDRF on vascular muscle. Although *in vitro* we have shown a relatively small difference in the response of muscle strips with and without epithelium, *in vivo* a similar effect might be magnified because of the geometry of the airways. Since resistance to air

flow increases in proportion to the fourth power of the radius of the airway so a small contraction of the circular muscle leads to a proportionally greater reduction in flow. Further characterization of this relaxant factor may lead to a greater understanding of the pathogenesis of asthma and be clinically important in the treatment of asthmatics.

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