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Exudation into the nasal cavity of carbon particles injected into nasal polyps*

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SUMMARY

A colloidal carbon solution was prepared by dissolving 50 mg of ultrafine carbon particles (diameter: 21-50 nm) and polyvinylpyrrolidone - for stabilizing the dispersion - in 1 ml physiological saline, and injected into the nasal polyps of allergic patients. Two hours after injection, the nasal polyps were removed and examined by transmission electron microscopy. Notably, carbon particles could not pass through the epithelial basement membrane and were therefore not observed between the epithelial cells, where no inflammatory cells infiltrated the epithelial layer. However, they passed through the fissure in the basement membrane, which was formed by the penetration of inflammatory cells (eosinophils) into the epithelial layer. Many carbon particles were observed in the interstitial space of the epithelial layer, where a large number of inflammatory cells accumulated. Furthermore, they passed into the nasal cavity along with the interstitial mucous fluid through the opened epithelial junction. A wide pathway from the submucosa to the nasal cavity, through which large-sized particles can pass, was demonstrated in the polyp's mucosa. Moreover, as carbon particles exhibit no chemotaxis, they must move according to the interstitial fluid flow, which suggests that the interstitial fluid flows outwardly from the mucosa during allergy.

Key words: macromolecules, nasal polyp, nasal allergy, epithelial basement membrane, epithelial permeability

INTRODUCTION

Rhinorrhoea is known to consist of a mixture of secreted products from the glands and/or goblet cells plus vascular products that leak into the lamina propria and then into the nasal cavity. Allergens (Raphael et al., 1991; Alkner et al., 1991) and histamine (Svensson et al., 1989; Raphael et al., 1989; Greiff et al., 1990; Alkner et al., 1991; Svensson et al., 1992), which are exposed on the human nasal mucosa, induce mucosal exudation of plasma macromolecules. It is now well established that inflammatory stimuli actively separate the endothelial cells in venules, and that infiltrated plasma exudates through the gaps of the microvascular wall. Raphael et al. (1989) have demonstrated that plasma albumin moves freely into the extravascular spaces and is distributed throughout the interstitium of the nasal mucosa following histamine stimulation. Also, plasma albumin has been demonstrated between the cells of the epithelium. However, it remains unclear how the macromolecules move across the epithelial lining and pass into the nasal cavity. Therefore, we have injected carbon particles - which are larger (diameter: 21-50 nm) than plasma macromolecules - into the submucosa of the nasal polyps, and have examined how this tracer passes through the epithelium and leaks into the nasal cavity, by means of electron microscopy.

MATERIAL AND METHODS

Five adult subjects affected by allergic rhinitis (3 women and 2 men; aged 20-35 years) were studied after informed consent was obtained. House dust was the causative allergen in all subjects, as determined by RAST. The patients received no treatment before removal of their polyps.

Colloidal carbon was prepared by dissolving 50 mg of Mitsubishi #40 ultrafine carbon particles (diameter: 21-50 nm) into 1 ml physiological saline. Polyvinylpyrrolidone was added to this solution to stabilize the dispersion (Hagiwara et al., 1984).

We injected 0.3-0.5 ml of the colloidal carbon into the subjects' nasal polyps. Two hours after the injection, the polyps were removed under general anaesthesia, and fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h at 4°C and then washed three times in 0.1 M cacodylate buffer (pH 7.4) contai-

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ning 8% sucrose. They were post-fixed in 1% OsO_4 in 0.1 M cacodylate buffer for 1 h at 4°C and then dehydrated with ethanol and embedded in Epon 812. Ultrathin sections were randomly cut from five different areas of each nasal polyp and examined by transmission electron microscopy.

RESULTS

Many carbon particles were distributed throughout the interstitium of the nasal polyp mucosa just beneath the epithelial basement membrane, but few of them were observed between epithelial cells where no inflammatory cells were obvious (Figure 1). The basement membrane apparently blocked their passage into the epithelial layer (Figure 2).

When the eosinophilic leukocyte moves into the subepithelial layer, it penetrates the epithelial basement membrane and carbon particles gather to the open space behind the eosinophilic leukocyte (Figure 3; arrowhead).

Carbon particles also had migrated into the epithelial layer following in the wake of the eosinophilic leukocytes (Figure 4), because the basement membrane was destructed by this penetration. In some areas, the basement membrane was not continuous, with many carbon particles clearly moving into the epithelial layer through these gaps (Figure 5; arrowhead). The gaps are thought to be formed by the penetration of inflammatory cells, although the basement membrane is perforated by nature. The mean number of inflammatory cells migrating into the basement membrane was 5.8 per mm of basement membrane, but the rate of migration varied between

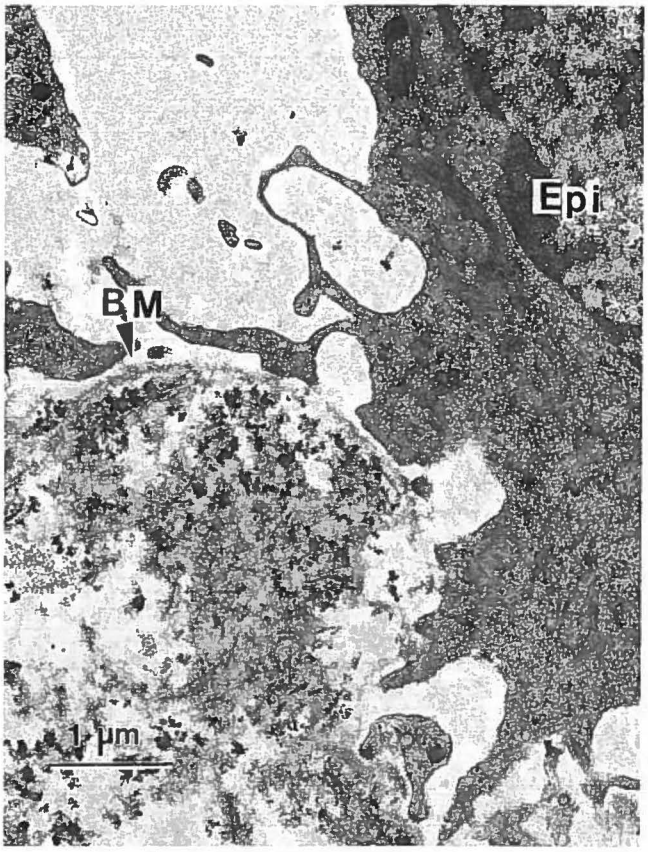

Figure 2. The basement membrane (BM) completely prevents penetration of carbon particles into the epithelial layer (Epi: epithelial cell).

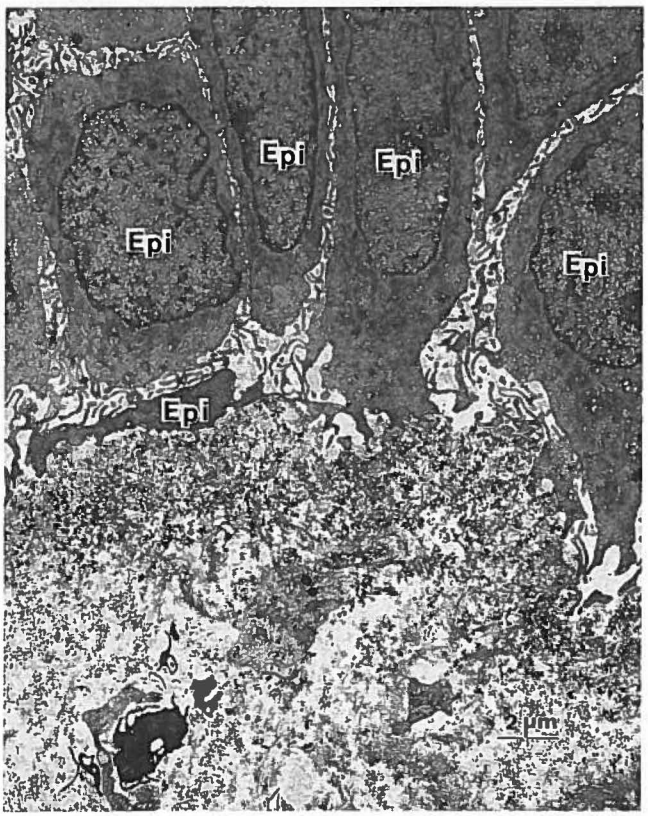

Figure 1. Carbon particles cannot pass through the epithelial basement membrane, and are not observed between the epithelial cells. No inflammatory cells are observed in this portion (Epi: epithelial cell).

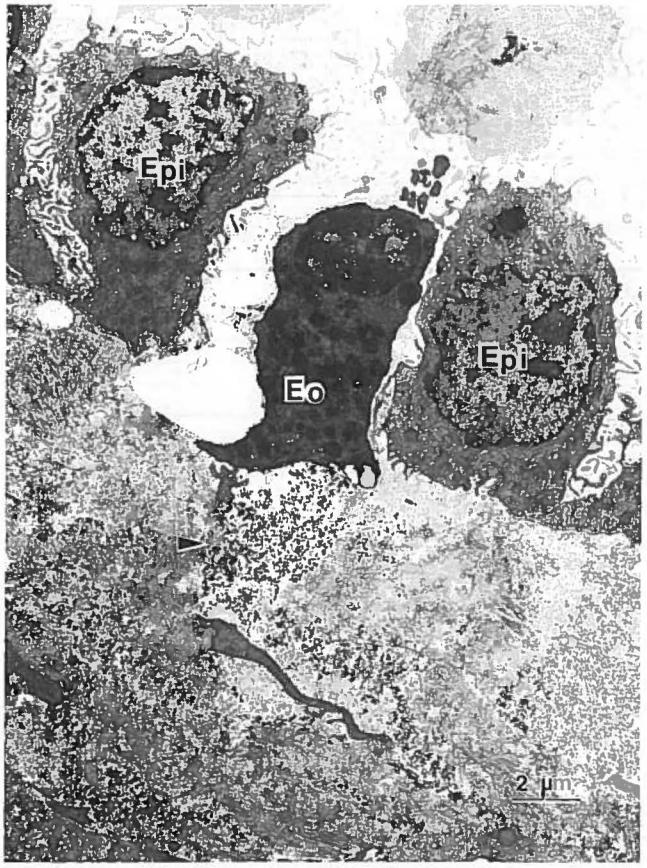

Figure 3. An eosinophil in the process of penetrating into the epithelial layer, with many carbon particles gathering behind it (arrowhead; Eo: eosinophil; Epi: epithelial cell).

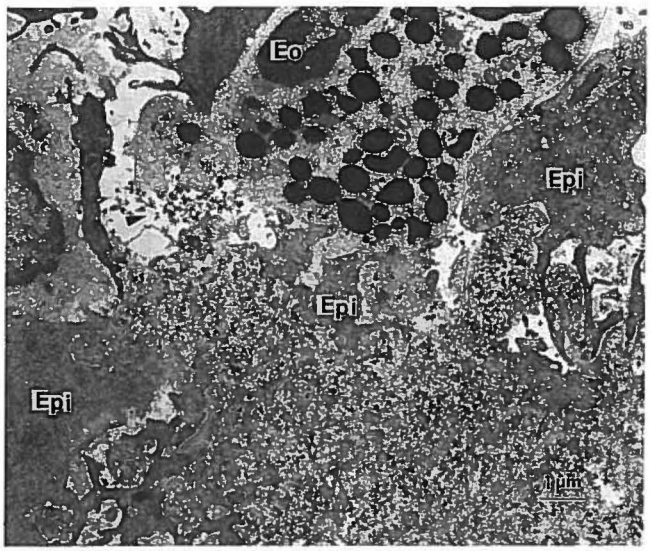

Figure 4. The epithelial basement membrane is destroyed when the eosinophil moves into the epithelial layer, with many carbon particles (arrowhead) passing into the epithelial layer through this fissure (Eo: eosinophil; Epi: epithelial cell).

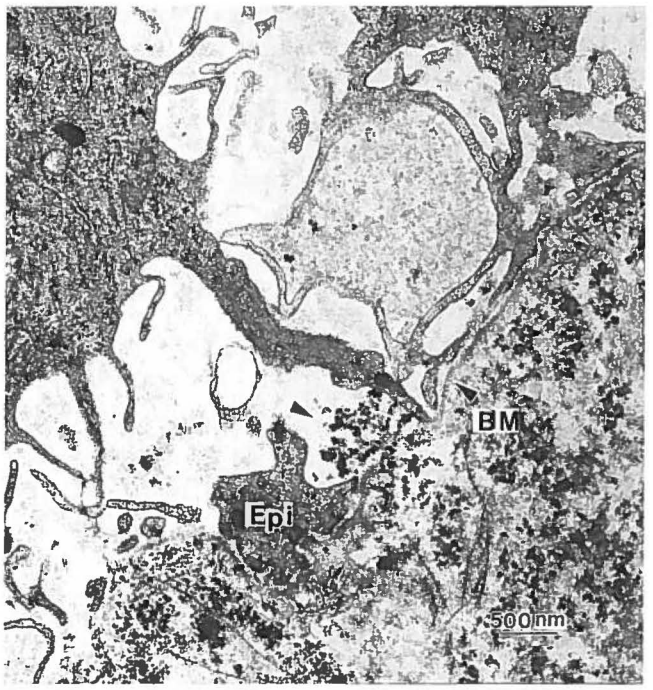

Figure 5. Carbon particles penetrate into the epithelial layer through the fissure of the basement membrane (arrowhead). This fissure is thought to result from eosinophil penetration (BM: basement membrane; Epi: epithelial cell).

subjects. The intercellular space between the epithelial cells was enlarged and many inflammatory cells infiltrated into this space, where many carbon particles were also observed (Figure 6). The enlargement of intercellular space was more conspicuous in the non-ciliated than in the ciliated area. The junctions between the epithelial cells were permeable and carbon particles leaked into the nasal cavity together with the interstitial mucous fluid (Figure 7). Leakage of carbon particles into the nasal cavity was frequently observed in the non-ciliated area, but only rarely in the ciliated area.

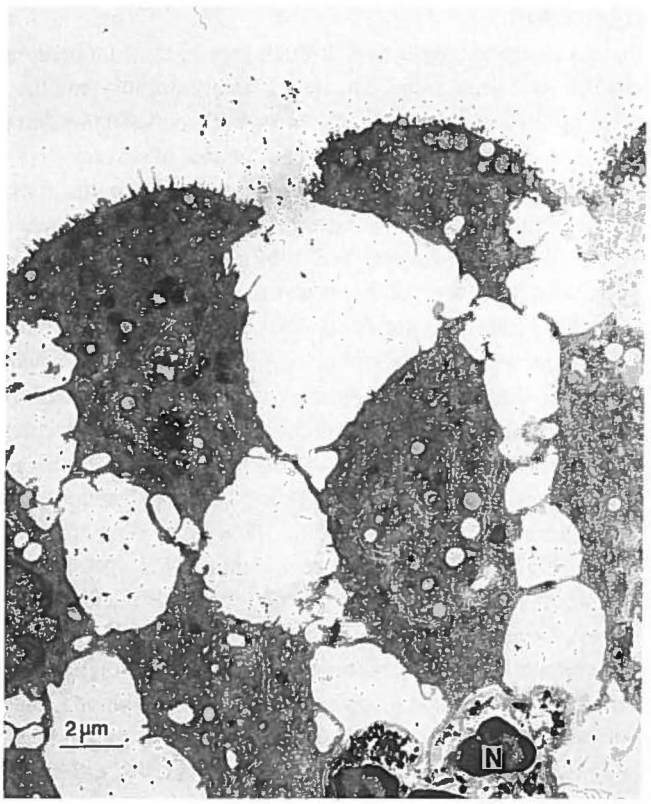

Figure 6. Many carbon particles and an inflammatory cell (neutrophil) are observed in the enlarged intercellular space. The epithelial cell is a non-ciliated cylindrical cell with microvilli (N: neutrophil).

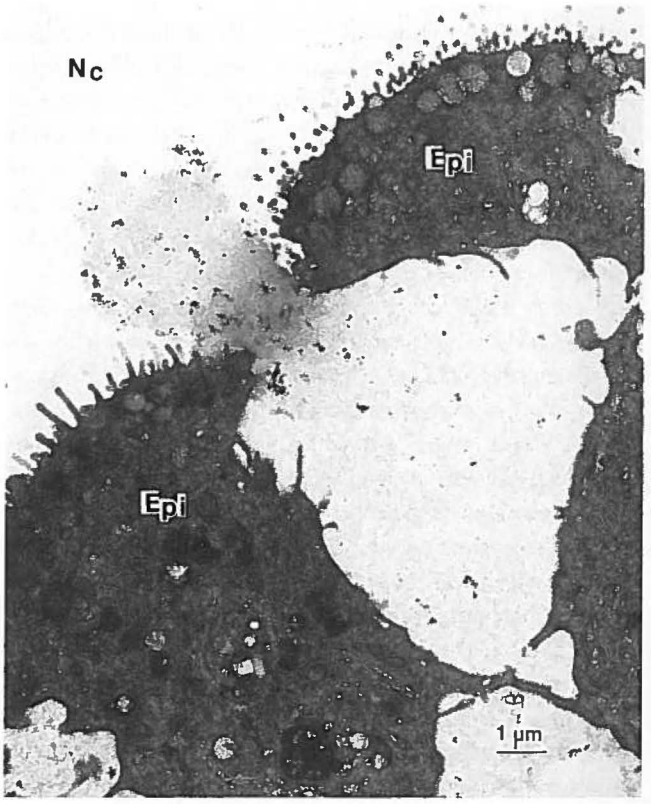

Figure 7. The cell junction between epithelial cells is separated. Many carbon particles leak into the nasal cavity along with the interstitial mucous fluid (Nc: nasal cavity; Epi: epithelial cell).

DISCUSSION

Plasma macromolecules pass through gaps in the endothelium - which have been induced by inflammatory stimuli - and they promptly leak through the nasal or tracheal epithelial layer into the lumen. The speed of passage and the lack of size selectivity in plasma macromolecule crossing strongly suggests that intercellular epithelial pathways have been opened (Persson et al., 1990). Notably, however, the mechanism underlying this movement and the route of plasma exudation across the epithelial lining has not been determined in detail. In this experiment we have used carbon particles as a tracer, which are larger in diameter than plasma macromolecules, such as albumin. Therefore, a large amount of plasma macromolecules certainly will leak into the nasal cavity along the same route by which carbon particles leak. Carbon particles cannot pass through the basement membrane of vessels (Clementi and Palade, 1969; Watanabe et al., 1980). Raphael et al. (1989) histochemically showed that albumin, which is smaller than colloidal carbon, freely pass through the basement membrane of vessels and epithelium in the nasal mucosa. Although carbon particles cannot move across the epithelial basement membrane, they did so when the inflammatory cells migrated into the epithelial layer and interrupted the basement membrane. In our study, carbon particles were observed in the large open intercellular spaces between the epithelial cells, although they could not be observed in the epithelial layer where there were few inflammatory cells (eosinophils). If the basement membrane would have many natural fissures, carbon particles would have be frequently observed in every intercellular space. It is thought that migration of inflammatory cells increases epithelial permeability. Erjefält and Persson (1991) reported that, after provocation by allergens, more plasma albumin leaks into the airway cavity than after provocation by bradykinin or capsaicin. The reason for this may be that inflammatory cells migrate only after the provocation of allergen, but do not after the provocation of bradykinin or capsaicin.

Luts et al. (1990) have demonstrated that epithelial tight junctions were ultrastructurally intact a few minutes after the passage of large volumes of plasma exudate into the tracheal lumen in response to several kinds of mediators. Additionally, Persson et al. (1990) assume that epithelial tight junctions reassemble immediately after separation. But plasma exudation through separated epithelial tight junction has not been demonstrated. In this regard, we have showed in this study that many carbon particles leak into the nasal cavity through separated epithelial junctions. Furthermore, this study has demonstrated that large-sized particles, such as colloidal carbon, leak into the airway lumen through undisrupted epithelium. Should macromolecules not leak into the airway lumen, this would lead to an increased osmotic load of the epithelium, resulting in shrinkage of the epithelial cells and subsequent desquamation. Desquamation occurs when oedema of the intercellular space is induced by factors such as allergen challenge. In our study, nasal polyps have been used. Although the mucous membrane of polyps differs somewhat from the nasal mucosa, it is possible that the epithelial junction also separates in the nasal mucosa since this characteristic of the mucous membrane of nasal polyps is very similar to the allergic nasal mucosa, in which accumulation of eosinophils, intercellular oedema, and thickening of the reticular lamina are observed.

It is considered implausible that the carbon particles themselves attract inflammatory cells, because few inflammatory cells were present in these areas where many carbon particles were observed (cf., Figure 1), and the degree of migration was almost the same as without the injection of carbon particles.

Carbon particles must move according to the interstitial fluid flow since they exhibit no chemotaxis. The carbon particles which have penetrated into the intercellular space between epithelial cells gradually move up to the surface and pass into the airway lumen through separated cell junctions. This suggests that the interstitial fluid flows outward from the allergic mucosa, and this supports the earlier findings of Persson et al. (1990). Their theory is that plasma exudate produces an increased hydrostatic pressure in the subepithelial interstitial space and its load transiently separates epithelial cells, providing a direction-selective and non-injurious intercellular pathway for passage of bulk plasma exudate into the airway lumen. It has been demonstrated in this study that exudation of macromolecules, such as carbon particles, into the airway lumen is caused by gaps in the epithelial basement membrane, as a result of inflammatory cells migrating into the epithelial layer and the separation of epithelial tight junctions by increased hydrostatic pressure.

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