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Macromolecular permeability of the tight junction of the human nasal mucosa

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SUMMARY

Using HRP as a cytochemical tracer, we investigated the tight junctional permeability of the human nasal mucosa in the early stage of its transportation.

We found that the tight junction becomes "leaky" in the inflammatory and in the allergic conditions. Connection of goblet-goblet and goblet-ciliated cells were weaker than that of ciliated-ciliated cells.

All epithelial tight junctions seemed to be loose in cases of allergic rhinitis.

INTRODUCTION

It has believed that the epithelial tight junction is impermeable. Morphological studies, using as a tracer horseradish-peroxidase (HRP), mol wt 40.000 daltons, suggest that the tight junctions ajoining the apical surface of epithelial cells act as effective barriers to restrict the permeation of protein into the submucosa (Inoue and Hogg, 1974; Richardson et al., 1973; 1976). However, respiratory mucosal permeability can actually be increased by IgE-mediated allergic bronchoconstriction (Boucher et al., 1977), and it is well known that stimuli, such as surgical trauma, electric shock, and cigarette smoke can cause the tight junction to open (Rhodes and Karnovsky, 1971; Hirano et al., 1970; Simani et al., 1974). In the present study, we investigated the tight junctional permeability of the human nasal mucosa using HRP as a cytochemical tracer.

MATERIAL AND METHODS

Thirty patients with a nasal disease were used in this study. From these thirty patients eight suffered from a septal deviation; they served as normal control group. Seven had non-atopic chronic sinusitis, eight had a nasal polyp accompanied by non-atopic chronic sinusitis and seven had allergic rhinitis.

10 mg of purified HRP (Toyobo; R.Z. 3.0) dissolved in 1 ml of 0.15 M NaCl was

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sprayed into the nasal cavity by a hand spray. After ten minutes, at the beginning of the surgery under deep NLA anesthesia, a specimen from the middle and/or inferior turbinates was taken by a small ear forceps and placed in 2.5% gluteraldehyde in 0.1 M phosphate buffer, pH 7.4. No topical medication was given. These specimens were subsequently sliced into blocks (approx. 1 × 2 mm) and fixed in the same fixative for 2 h at 4 °C. From each patient about 10 blocks were prepared and they were used for localization of peroxidase activity by the method of Graham and Karnovsky (1966) and Adams (1981). These blocks were further fixed in 1% osmium tetraoxide in 0.1 M phosphate buffer, pH 7.4 for 1 h at 4 °C. They were then dehydrated by means of a series of graded ethanol in a Quetol 812. In order to investigate the endogenous peroxidase activity, specimens were obtained from four patients from each group. In these cases, HRP was not sprayed into the nasal cavity, but the specimens were incubated in the same fixation and the DAB reaction. Thick (1-μm) sections were prepared from each block and stained with toluidine blue for light microscopy. Blocks that had a suitable amount of the tracer reactions were selected for further processing. Ultra-thin sections from such blocks were cut and stained with uranyl acetate and lead citrate. These sections were examined by a JEM 100 B electron microscope. Only junctions that showed a reaction product on the luminal surface of both adjacent cells were evaluated for penetration of the intercellular space by HRP and the number of junctions fulfilling these criteria was counted.

RESULTS

No peroxidase activity could be detected in the junctional region and in the intercellular space of the control subjects in each group. The reaction product of HRP was observed in the periciliary space of the nasal epithelium from subjects with septal deviation. In these subjects the reaction product was rarely found in the intercellular space and in the ciliated and goblet cells (Figures 1 and 2). Most of the reaction product of HRP accumulated on and between the cilia of the nasal epithelium of chronic sinusitis. No uptake of HRP was observed in their epithelial cytoplasm (Figure 3) but sometimes, tracer was found in the intercellular space between a goblet cell and a ciliated cell (Figure 4).

There were many sites distinctly showing the presence of the reaction product in the intercellular space between a goblet cell and a ciliated cell, or between goblet cells in the epithelium of the polyp accompanied by chronic sinusitis. The tracer, however, was seldom found in the intercellular space between ciliated cells (Figure 5). An abundant reaction product was found in the intercellular space of nasal epithelium from patient with allergic rhinitis. Where an abundant reaction product was present, HRP penetrated particularly into intercellular space between ciliated cells (Figure 6). There was a tendency of the reaction product to gradually decrease at the site near the basal part of the epithelium (Figure 7). It

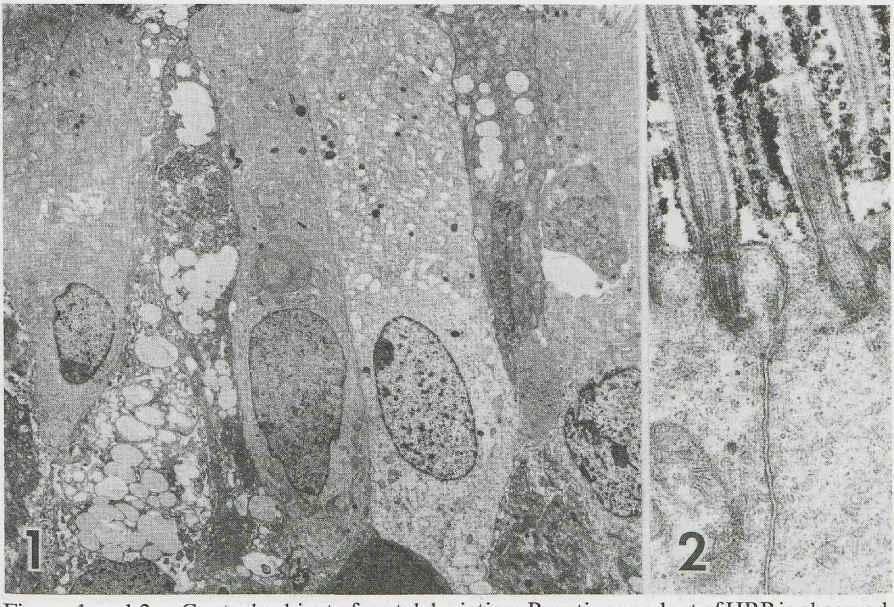

Figure 1 and 2. Control subject of septal deviation. Reaction product of HRP is observed in the periciliary space, but is not found in the intercellular space ($\times$2,500, $\times$25,000).

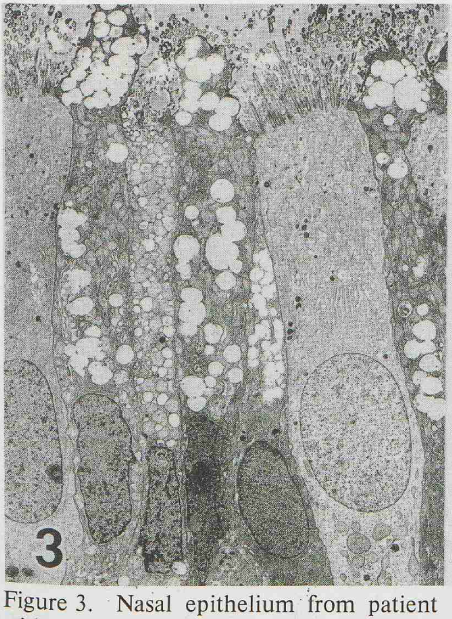

Figure 3. Nasal epithelium from patient with chronic sinusitis. Reaction product of HRP is accumulated on and between the cilia. No uptake of HRP is found in these epithelial cells and intercellular space ($\times$2,500).

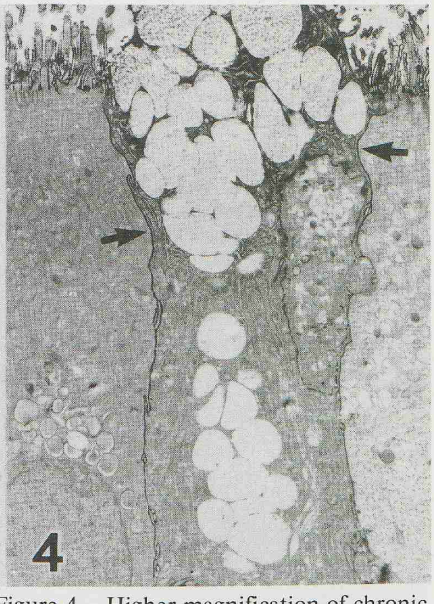

Figure 4. Higher magnification of chronic sinusitis. In this case HRP is found in the intercellular spaces between a goblet cell and a ciliated cell (arrows), ($\times$3,500).

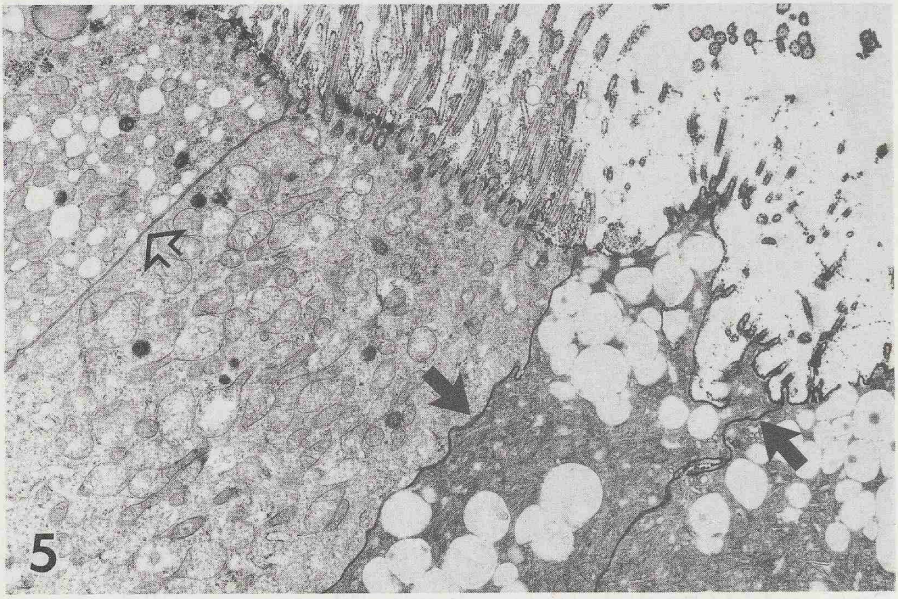

Figure 5. Nasal polyp from patient with non-atopic chronic sinusitis. Intercellular spaces between a ciliated cell and a goblet cell or between goblet cells are filled with reaction product (close arrow), but little tracer is found in the intercellular space between ciliated cells (open arrow), ($\times$ 5,100).

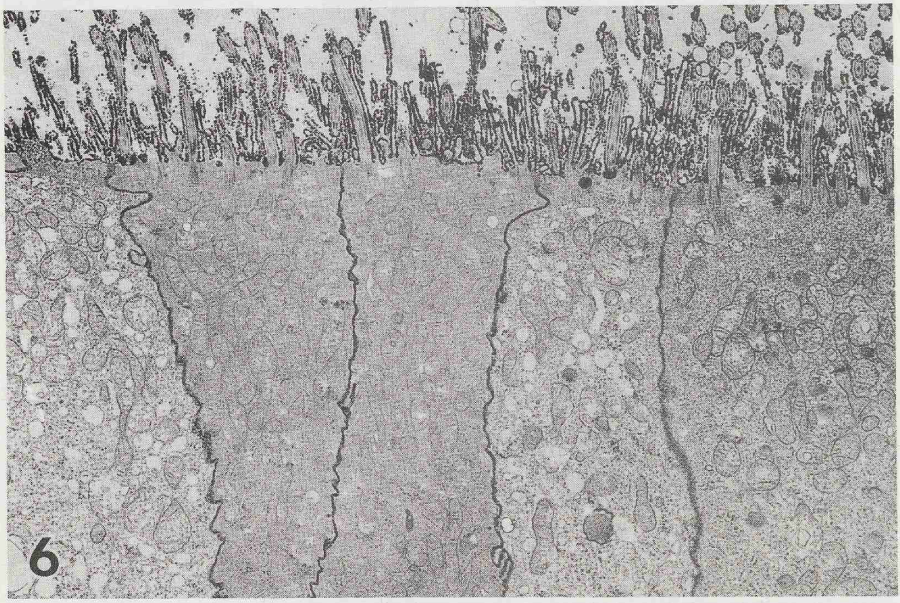

Figure 6. All intercellular spaces and tight junctions are filled with reaction product at the inferior turbinate of patient with allergic rhinitis ($\times$ 5,400).

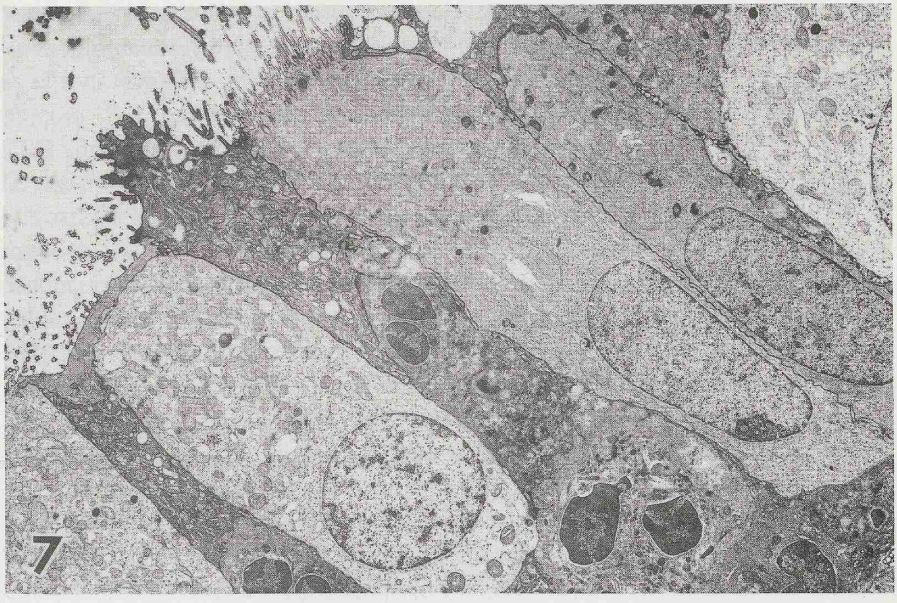

Figure 7 . Reaction product gradually decreases at the site near the basal part of the epithelium of patient with allergic rhinitis (× 3,300).

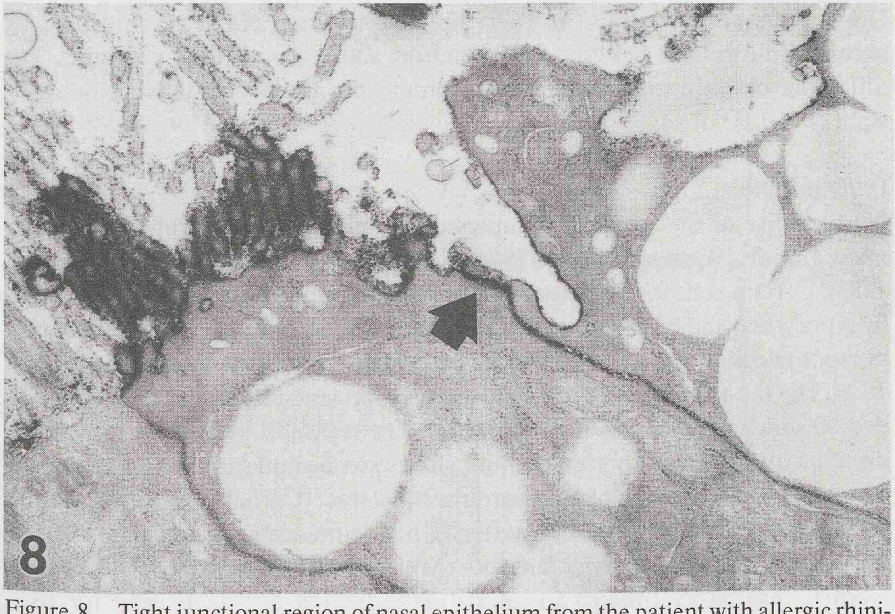

Figure 8. Tight junctional region of nasal epithelium from the patient with allergic rhinitis. HRP penetrates into the tight junction (× 15,000).

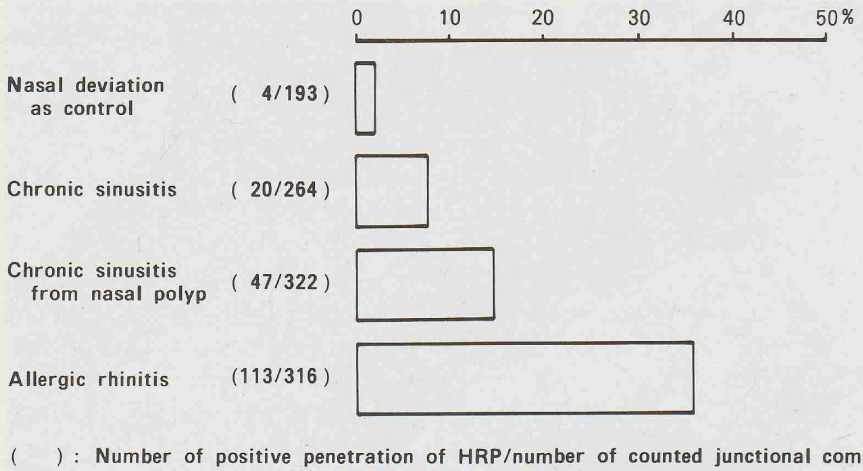

Figure 9. Total number of junctions from each group examined and judged om HRP penetration.

was obvious that HRP penetrated into the tight junction (Figure 8).

Figure 9 shows the total number of the tight junctions examined from each group and the number judged to be penetrated by HRP. The ratio of penetrated HRP to the total number was 4 to193 (2.1%) from subjects with septal deviation, 20 to 264 (7.6%) from chronic sinusitis, 47 to 322 (14.6%) from polyp accompanied by chronic sinusitis and 113 to 316 (35.8%) from subjects with allergic rhinitis. The difference of the junctional penetration among the groups are statistically significant ($p < 0.1$).

DISCUSSION

Permeability of the respiratory mucosa has been studied morphologically by many authors (Richardson et al., 1976; Nakai, 1975; Masuda, 1983; Inagaki et al., 1984). Certain cells in the mucosa of the respiratory tract take up tracer proteins by pinocytosis and transport them either to the lateral cell wall or to the basal portion of the cell where they are then released into the extracellular space (Figure 10.1). The time required for this uptake, transport, and release varies between 30 and 60 minutes (Richardson et al., 1976). The transport system for exogenous proteins in the respiratory epithelium shows some similarities to the transport system in the epithelium of the gastrointestinal tract (Cornell et al., 1971; Hugon and Borges, 1968). Such transportation of macromolecular substances is known as energy-dependent active transport (Miyoshi, 1971; Sakakura et al., 1983). Normal respiratory mucosa in animal experiments has been used for such studies. There is a possibility that another transportation route may exist in the

abnormal or pathological nasal mucosa in man. We investigated the tight junctional permeability of the human nasal mucosa of a pathological condition, such as chronic sinusitis and allergic rhinitis, and compared it to the normal nasal mucosa. The nasal mucosa was obtained ten minutes after having administered HRP and thus a very early stage of permeation would be observed.

In the preliminary experiment endogenous peroxidase activity was examined (Christensen et al., 1981), and in the present study no peroxidase activity was found in the junctional complex, or in the intercellular space. The reaction product of HRP was found in the intercellular space between a ciliated cell and a goblet cell in the polyp accompanied by chronic sinusitis. However, the uptake of HRP into the epithelial cell was not observed. There were more goblet cells in the mucosa of the polyp, so that the number of the tight junctions through which HRP permeated was more than in the case of the septal deviations or the inferior turbinate from chronic sinusitis. Generally, there was more reaction product in the intercellular space between the ciliated-goblet cells or goblet-goblet cells than that between ciliated-ciliated cells. These facts suggest that the connection between a ciliated cell and a goblet cell or between goblet cells mutually are relatively loose, and that the connection between ciliated cells are tight. Inoue et al. (1977) examined the tracheal epithelium of normal guinea pigs by the freeze-fracture technique, and showed that the tight junctions between ciliated cells were well developed, but the development was not uniform between a ciliated cell and a goblet cell. Thus, the tight junction at this site is "leaky". This present study is well in agreement with Inoue's observation. The tight junction is not functionally stable.

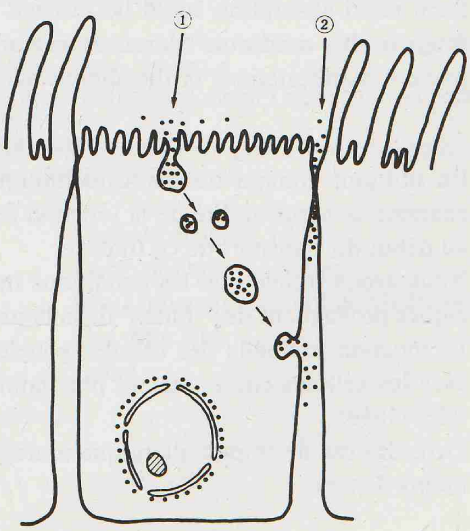

Figure 10. Absorption pathway of macromolecualr substances through the nasal mucosa in the normal (1) and in the pathological (2) condition.

There were many sites in which the reaction product was observed in the intercellular space in the inferior turbinate from patients with allergic rhinitis. The amount of HRP penetrating the tight junction was greater than that of other three conditions. Thus, all epithelial junctions seemed to be loose in cases of allergic rhinitis (Figure 10.2). Using the tracheal epithelium of guinea pigs, Boucher et al., (1978) found by an electron microscopic observation that the absorption of HRP increased after the administration of histamine. It may be that the connection of the epithelial cells in human nasal epithelium is disturbed and loosened by various chemical mediators in the allergic condition. In the present study, in one specimen taken from a patient with seasonal rhinitis during an off season the number of junctions through which HRP permeated was 17%. This ratio is lower than the average in allergic rhinitis, but higher than those of the control and chronic sinusitis.

The junctional complex serves as the protective mechanism of the nasal mucosa together with the mucociliary transport system. However, all epithelial junctions do not have the same connections. The connections of the goblet-goblet and goblet-ciliated cells are weaker than that of the ciliated-ciliated cells. Therefore an increase in the number of goblet cells results in an enhancement of epithelial permeability. In the allergic condition, the tight junction loosing its protective function allows the permeation of macromolecular substances. It is possible that the reduced barrier function of the tight junction could be the result of damage to epithelial cells caused by the chemical mediator.

We found that the trans-junctional penetration took place within ten minutes after the administration of HRP. This is extremely faster than trans-cellular penetration and may be a route by which allergens penetrate into the nasal mucosa. Such rapid absorption could be utilized for topical administration of various drugs in this condition. Moreover, closure of the loosened tight junction may prevent manifestations of the allergic symptoms.

RÉSUMÉ

En utilisant comme traceur cytochimique la peroxydase (HRP),nous avons examiné la perméabilité de la jonction dense de la muqueuse nasal humaine, au début du transport de ce traceur.

Nous avons trouvé que les conditions inflammatoires et les conditions allergiques provoquent des "fuites" dans la jonction dense.

La jonction mutuelle des cellules gobelet et la jonction des cellules gobelet avec les cellules ciliées étaient plus faibles que la jonction mutuelle des cellules ciliées.

Dans des cas de rhinite allergique toutes les jonctions épithéliales semblaient moins denses.

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