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Nasal absorption in the rat. I: A method to demonstrate the histological effects of nasal formulations

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Summary

A method is described which enables the controlled assessment of the effects of intranasal drug formulations on rat nasal epithelium. Damage to the nasal mucosa caused by the surfactant laureth-9 (1% w/v) was used as an indicator of the distribution of dose volumes (100, 50 and 20 μl) administered to one side of the nasal cavity. Only the 20 μl volume was retained exclusively in the dosed side. Using this dose volume therefore, the epithelium on the undosed side could be used as control tissue and a direct comparison made between experimental and control tissue in the same section. Errors due to inter-animal variablity and tissue sampling are thus minimised, and the total number of animals required for future studies reduced.

Introduction

Nasal drug delivery is commonly associated with the topical treatment of local conditions such as allergic rhinitis and symptoms of the common cold. Recently, however, there has been a growing interest in the nasal route for the systemic delivery of drugs which have low oral bioavailabilities because of degradation in the gastrointestinal tract or extensive mucosal or hepatic metabolism. Enzymatic degradation is a particular problem when considering the oral delivery of therapeutically active peptides and proteins (Banga and Chien, 1988) and in many cases (e.g. insulin, human growth hormone) the only efficient route of administration currently available is by injection. The pain associated with injections tends to result in low patient acceptability and when chronic therapy is necessary, careful patient education and training is essential to enable self-administration and avoid possible complications.

In contrast, the nasal route is easily accessible and has good patient acceptability. However, in common with other non-parenteral routes such as rectal, vaginal and buccal, absorption of many peptide and protein drugs across the nasal mucosa is relatively low. Increased absorption has been achieved in a number of studies by increasing the dose of drug (Chien, 1985), by reducing clearance mechanisms in the nose with mucoadhesive systems (Illum et al., 1987) and by the co-administra-

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tion of absorption enhancers such as: surfactants, including bile salts and their derivatives, (Duchateau et al., 1986, 1987; Longenecker et al., 1987; Wheatley et al., 1988); chelators (Cho et al., 1989) and enzyme inhibitors (Aungst and Rogers, 1988).

Many absorption enhancers are irritant to mucous membranes (Waller et al., 1988; Richardson et al., 1989) and the histological effects of some agents on nasal mucosa have been reported by other researchers. Lee et al. (1988) described cellular erosion, cell-cell separation, dense mucous coating and loss of cilia from rat nasal epithelium five minutes after the administration of the surfactants laureth-9 (1% w/v) or sodium deoxycholate (1% w/v). Hersey and Jackson (1987) observed loss of surface epithelium from rabbit and dog nasal mucosa following exposure to 0.5% w/v sodium deoxycholate in vitro. The destructive effects of 1% w/v laureth-9 were also noted by Daugherty et al. (1988), when administered with methionyl-human growth hormone (Met-hGH), including complete removal of the epithelium in some regions; similar histopathology was seen 30 min and 24 h after nasal dosing.

Young (1981) has made a detailed examination of the rat nasal cavity and described the variability in epithelium types lining the anatomical structures. Experiments investigating the histological effects of enhancers have usually used untreated animal as control. In view of the complex nature and distribution of the tissue, a better animal model would allow not only absorption studies with histological sampling at the end point, but also control tissue sampling from the same animal.

The aim of this study was to establish whether the anatomical division of the rat nasal cavity by the midline septum might allow the two halves to be treated as experimental and control surfaces for the assessment of the histological effects of absorption enhancers, as a prelude to combined absorption and histological studies. To this end, rats were dosed nasally; (a) with a known irritant (laureth-9, 1% w/v), to establish that epithelium disruption could be detected; (b) with different dose volumes of this test solution, to investigate dose distribution in the nasal cavity and in particular the possibility of leakage via the septal 'window' in more posterior regions.

Materials and Methods

Materials

Polyoxyethylene-9-lauryl ether (laureth-9) was purchased from Sigma (Dorset, U.K.). Standard histological preparations and all other materials used were of reagent grade.

Animal preparation and dosing

Male Wistar rats (JABU, Sutton Bonington, U.K.) of approx. 250 g and anaesthetised intraperitoneally with 60 mg/kg sodium pentobarbitone (Sagatal, 60 mg/ml, May and Baker, Essex, U.K.) were used for all investigations.

In the test groups, a tracheotomy was performed on each animal prior to the nasal administration of the formulation, as described by Illum et al.(1989). To prevent drainage of the dose from the nasal cavity the oesophagus was ligatured onto the tracheal cannula. The test solution was delivered to the right nostril only, using a Hamilton syringe with attached length of polyethylene tubing; the dosing tube was inserted about 0.5 cm into the nostril. Animals remained supine throughout the experiment.

Completely undosed control tissue was obtained from animals killed by an overdose of anaesthetic, without any prior cannulation.

Dosing experiments

Groups of three animals were dosed with 1% w/v laureth-9 (polyoxyethylene-9-lauryl ether) solution in phosphate buffered saline. To investigate dose distribution in relation to dose volume animals received volumes of either 100, 50 or 20 μl. To investigate the possibility of leakage of the dose into the undosed side, within each volume group rats were perfusion fixed 5, 20 or 60 min after dosing.

Tissue preparation

Following a 2 min prewash by cardiac perfusion of buffer solution (containing a vasodilator) tissue was fixed by perfusion of Bouin Hollande fixative for approx. 15 min. The animals were decapitated, the mandible, brain and excess soft tissue removed. The specimens were placed in fresh fixative baths overnight. Specimens were de-

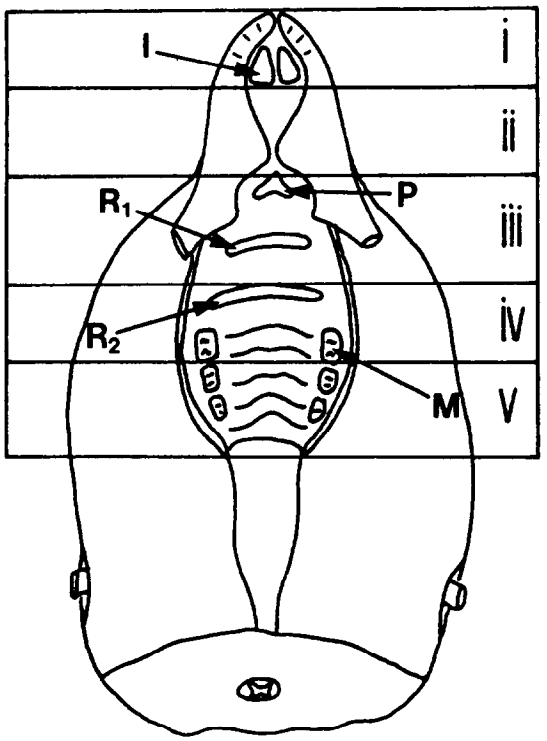

Fig. 1. Diagram illustrating the surface features of the roof of the oral cavity in the rat. Those landmarks used to define cutting positions for the division of the nasal cavity into regions (i)–(v) are indicated: upper incisor root (I); incisive papilla (P); 1st palatal ridge (R_1); 2nd palatal ridge (R_2); 1st upper molar (M).

calcified in 20% EDTA solution (adjusted to pH 7.0) for 3–4 weeks by which time it was possible to divide each into 5 regions (Fig. 1), adapting the method used by Young (1981). All regions were processed through to paraffin wax blocks using routine histological methods. Each region was orientated in the wax so that complete transverse cross-sections of the nasal cavity were produced, with the anterior face presented for cutting first.

Sections were cut serially at 7–8 μm thickness, mounted and stained with haematoxylin and eosin. Acidic mucopolysaccharides were demonstrated with alcian blue (Preece, 1972) and neutral mucopolysaccharides with periodic acid/basic fuchsin. (Horobin and Kevill-Davies, 1971). Cross-sections of the nasal cavity were examined with the light microscope.

The appearance and distribution of normal rat nasal epithelium were identified in sections from completely undosed control animals. In each section from regions (ii)–(iv) of test animals, comparisons were made between the dosed and undosed sides of the nasal cavity and also between the undosed side of these sections and corresponding sections from undosed control animals.

Results

The major structural features in each region of the rat nasal cavity were identified in the cross-sections produced, including the septal window connecting the two sides of the cavity (Fig. 2).

In sections prepared from undosed animals, a continuous epithelial layer covered all surfaces of the rat nasal cavity. Keratinised stratified squa-

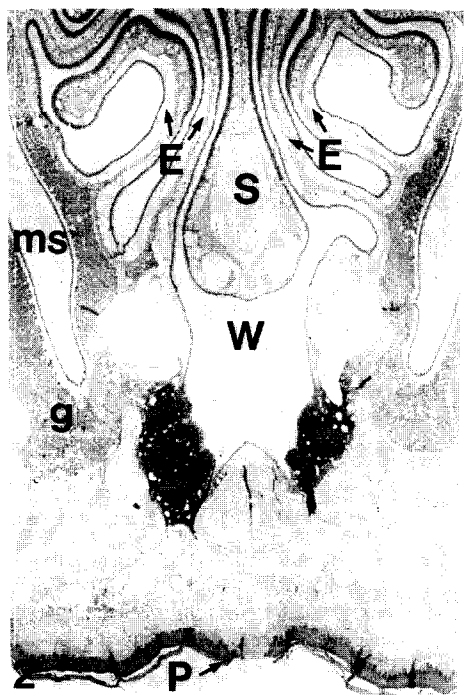

Fig. 2. A photomicrograph of a vertical section through region (iv) of the rat nasal cavity illustrating the septal window (W), a discontinuation in the nasal septum (S) which allows exchange between the left and right sides. Also indicated are the ethmoid turbinates (E), the maxillary sinuses (ms), glandular tisue (g), tonsilar tissue (t) and the hard palate (P) (H.E. ×30).

mous epithelium covered surfaces in region (i) and extended into the ventral meatus of each side of the nasal cavity in region (ii). In region (ii) the septum was covered with typical respiratory epithelium, i.e., ciliated pseudostratified columnar epithelium densely populated with mucus filled goblet cells. The epithelium on the anterior turbinate surfaces was pseudostratified cuboidal in form, it was only sparsely ciliated and contained few goblet cells (Fig. 3).

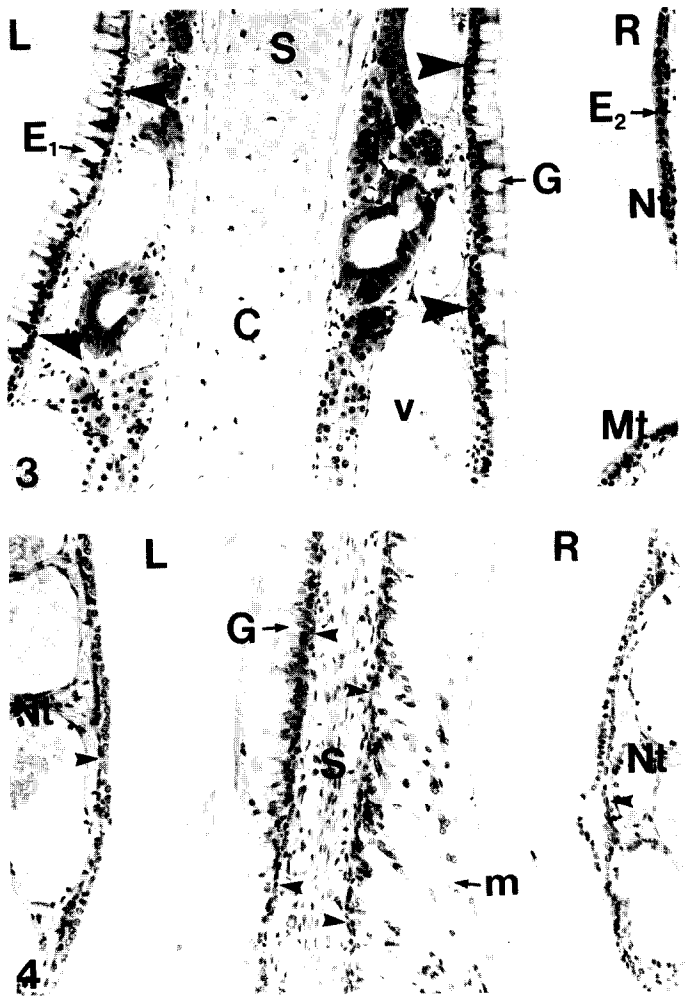

Fig. 3. A photomicrograph of a vertical section from region (ii) of the rat nasal cavity showing the left (L) and right (R) sides. The nasal septum (S) contains cartilage (C) and is covered by ciliated pseudostratified columnar epithelium (E_1) densely populated with pale staining, mucus filled goblet cells (G). Glandular tissue (g) and vascular sinuses (v) are present in the subepithelial layer (lamina propria). The sparsely ciliated pseudostratified cuboidal epithelium (E_2) covering the nasoturbinate (Nt) and maxilloturbinate (Mt) is also shown (H.E. ×255). Note: arrowheads (›) indicate the junction of the epithelium with the lamina propria in this and subsequent photomicrographs.

Fig. 4. A photomicrograph of a vertical section from region (ii) of the rat nasal cavity 5 min after dosing 100 μl of 1% w/v laureth-9 solution into the right side (R). Epithelium disruption has occured on septal (S) and nasoturbinate (Nt) surfaces, particularly on the dosed side. Discharged mucus, containing cells shed from the epithelium, is present in the lumen of both sides of the cavity (m) but some intact goblet cells (G) remain in the left, undosed side (L). The photomicrograph shows an area of the section in which the septum is without cartilage; this indicates that it was sampled from the anterior part of region (ii) (H.E. ×255).

Transitions to olfactory epithelium occurred in more posterior regions of the cavity, particularly in region (iii); in these transitional areas epithelium height was more variable. Olfactory epithelium was present throughout the ethmoid recess in regions (iv) and (v).

Thus, several types of epithelium were identified throughout the nasal cavity. Epithelium of a particular type was not always consistent between animals, particularly with respect to epithelial height and sites of transitional zones. Importantly however, the epithelium on the left and right sides of the nasal cavity in each cross-section matched each other with respect to the distribution of the different epithelium types and epithelial height. The respiratory epithelium of the nasal septum

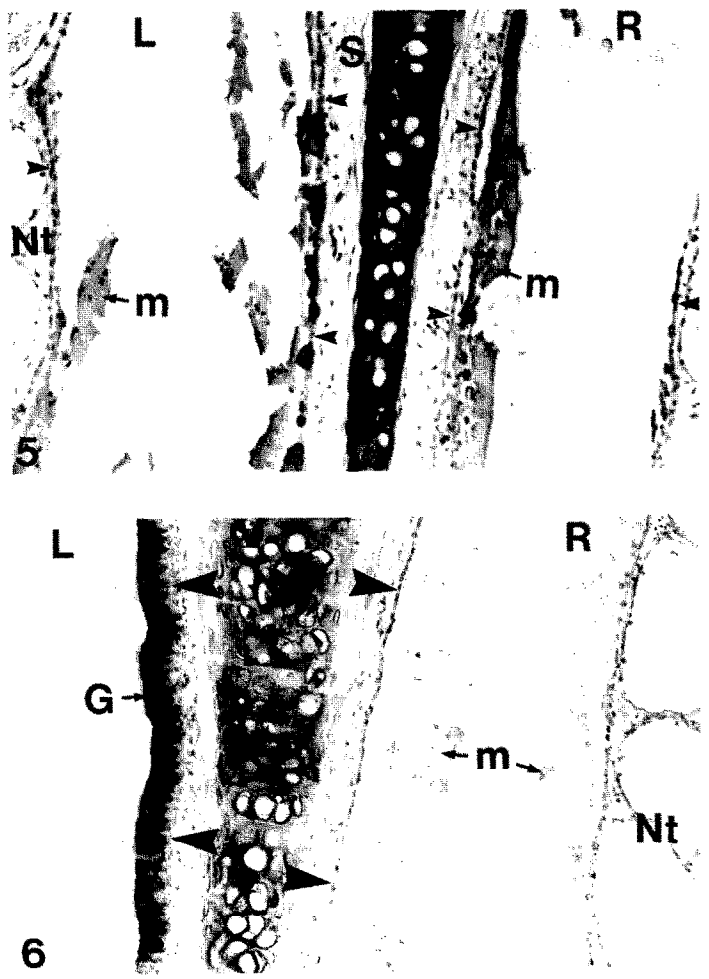

Fig. 5. A photomicrograph of an alcian blue stained vertical section from region (ii) of the rat nasal cavity 1 h after dosing 100 μl of 1% w/v laureth-9 solution into the right side (R). Acidic mucopolysaccharides in mucus (m) and cartilage (C) have taken up the darker alcian blue stain. Few goblet cells remain intact and the respiratory epithelium is severely reduced in height on both the dosed (R) and undosed (L) sides of the cavity ($\times$255).

Fig. 6. A photomicrograph of an alcian blue stained vertical section from region (ii) of the rat nasal cavity 1 h after dosing 20 μl of 1% w/v laureth-9 solution into the right side (R). Respiratory epithelium on the septum (S) and nasoturbinate (Nt) in the dosed side has been reduced to a single layer of cells; discharged mucus (m) containing lost epithelial cells is present in the lumen. On the undosed side (L) the respiratory epithelium remains undisturbed with goblet cells (G) full of alcian blue staining mucus ($\times$255).

appeared to be the most consistent in composition over a relatively large area.

Epithelium exposed to the 1% w/v laureth-9 solution exhibited sites of interaction including epithelium disruption, cell loss and subsequent reduction in epithelium height (Figs 4 and 5). Complete loss of epithelium occurred in some places and large quantities of mucus were observed free in the nasal cavity. Only the keratinised stratified squamous epithelium in the ventral meatus showed no changes when compared to the control tissue from undosed animals. Increased time of contact emphasized the effects of the surfactant and damage was more widespread. The epithelium disruption was easily identified under the light microscope and was therefore used as the indicator of surfactant contact and dose distribution.

The effects of 100 μl of the 1% w/v laureth-9 solution were observed in both sides of the nasal cavity 5 min after administration, though increased cell loss and mucus discharge had occurred on the dosed side. Epithelium disruption on the undosed side was severe but less widespread, with most sites of interaction present around the septal window (Fig. 4). Increased time of contact increased the distribution of epithelium disruption especially on the undosed side of the cavity (Fig. 5). The specific stains applied identified large quantities of mucus of mixed composition, free in both sides. Olfactory epithelium in the ethmoid recess was disrupted so that the epithelial boundaries could not be clearly identified. Damage extended over the septal tip and around the septal window at all of the contact times investigated.

After 5 min a 50 μl volume of the dose solution had resulted in similar widespread and severe epithelium disruption in the more anterior regions of the dosed side. The distribution of damage in the dosed ethmoid recess was variable and some ethmoid turbinate surfaces were unaffected. There were few effects on the undosed side after 5 min except in the area immediately around the septum tip but by 20 min mucus was present in the lumen and some cell loss had occurred proximal to the septal window. After 60 min increased amounts of cell loss and mucus discharge had occurred on the undosed side though sites of epithelium interaction were generally more sparsely distributed than on the dosed side and relatively large areas of septal epithelium remained intact. Mucus was observed around the undosed ethmoid turbinates but epithelium disruption only occurred in more ventral areas of the recess on this side.

Epithelium interactions following the administration of the 20 μl dose volume were further restricted in distribution. On the dosed side, extensive cell loss, mucus discharge and epithelial cell rearrangement was obvious in all sections from regions (ii) and (iii) irrespective of contact time; effects were limited to the septal and ventral areas in the more posterior sections. Epithelium throughout region (iv) of the dosed side was unaffected except for slight mucus discharge.

The epithelium around the septal window which connects the two sides was undamaged, with mucus filled goblet cells present over the septal tip. Moreover, in each animal administered with 20 μl, all regions of the undosed side of the nasal cavity were covered by an intact, undamaged epithelium layer, comparable to that observed in sections prepared from completely undosed control animals (Fig. 6).

Discussion

To enable comparison of the effects of different enhancer agents on the nasal membrane, some method of assessing the degree of histological change must be applied. Qualitative assessment may be possible if gross damage has occurred, but knowledge of the normal epithelium state is still necessary; hence, a histological control is required. Treated epithelium has to be compared with control epithelium of the same type. Identification of epithelium type however, is not always simple when it has been damaged, therefore the use of separate controls relies heavily on precise selection and matching of sections for comparison.

Even if careful sampling is possible, the variability found in the distribution of nasal epithelium in the rat nasal cavity still makes prediction of epithelium type in a particular section difficult. Positions of certain anatomical features such as the turbinates, are likely to be subject to a similar

degree of variation, adding to the problem. Epithelium distribution however, was found to be consistent between the two halves in the same transverse plane of the cavity so that direct comparison of the same epithelial type is possible between matched sample points on the left and right sides in the same section. Such a comparison therefore negates problems associated with inter-animal variability and accurate tissue sampling between animals.

Laureth-9 1% w/v was found to be an effective irritant of rat nasal mucosa with changes apparent after 5 min, in agreement with published reports (Lee et al. 1988). The epithelium damage resulting from contact with the surfactant solution was easily identified and was therefore used as the indicator of dose volume distribution.

In the present study a dose of 100 μl was found to distribute widely in both sides of the nasal cavity even 5 min after dosing. Damage around the septal window indicated contact with the surfactant in this area, suggesting that leakage occurred into the undosed side of the cavity by this route. This is in contrast to recent results published by Tengamnuay and Mitra (1990). They also considered the use of the undosed side of the cavity as control after observing unilateral lesions when 80–100 μl of a solution containing bile salt and fatty acid mixed micelles was administered to one nostril only. These results are surprising considering that most of the histological changes were observed in olfactory epithelium in posterior regions of the cavity where the septum dividing the two sides is incomplete. The septal window was not mentioned as a potential problem.

In this latest investigation, a dose of 50 μl was more restricted in distribution, particularly in the ethmoid recess, but still 'leaked' into the undosed side where epithelium proximal to the septal window was the more severely affected.

It is unlikely that the effects in the undosed side were due to the diffusion of surfactant solution across the nasal septum from the dosed side. The dense septal cartilage presents an effective barrier to diffusion and dose solution is unlikely to have penetrated the septum and lamina propria (sub-epithelial layer) in sufficient quantity and in the time allowed to reach the epithelium and cause the considerable disruption that was observed. The close relationship between dose volume and extent of epithelium disruption on the undosed side of the nasal cavity also supports the theory of leakage via the septal window and further encourages the possibility of using undosed epithelium as control tissue when the dose volume is restricted.

Instillation of 20 μl was shown to be contained in the dosed side of the nasal cavity only, even 1 h after administration. The septal window area and all of the undosed side of the nasal cavity was clear of epithelium damage. The supine position of the animal, as well as the reduced volume, would contribute to this restricted distribution; the dose solution would tend to flow downwards into dorsal areas and away from the septal window. Occasionally, an air lock or mucus in the narrow space between septum and turbinates appeared to prevent flow into the dorsal meatus, judging by the lack of effects in such areas, but the small dose volume still prevented leakage into the undosed side.

The effects of 20 μl of the surfactant solution were widespread in the dosed half of region (ii) suggesting good distribution and contact with most surfaces, thus providing a reasonable area for drug absorption and histological sampling and analysis. This dose volume resulted in more variable distribution in region (iii), particularly in more posterior areas. Exposure of all epithelium in this region to 20 μl of test solution cannot be assumed therefore.

Restricting the analysis to region (ii) would not detect the effects of agents on olfactory epithelium. The area covered by olfactory epithelium in man however (approx. 20%) (Pontiroli et al., 1989), is much less than in the rat (50%) (Gross et al., 1982) which relies more heavily on its sense of smell for survival (Negus, 1958). Respiratory epithelium, required for air conditioning, is of major significance in man (approx. 80%) and the effects of nasally administered agents on this type of mucosal surface are perhaps more important in the initial stages of investigation; location of the 47% of rat respiratory epithelium in the anterior regions of the cavity as described, enables such analysis. The effects on olfactory epithelium may be investigated in follow up studies if required,

although a separate group of undosed control animals would be needed as leakage into the undosed side would be unavoidable when targetting tissue in the most posterior parts of the cavity. Septal respiratory epithelium in region (ii), densely populated with goblet cells, was found to be particularly consistent in composition. Use of such a uniform test surface would further increase confidence in the results obtained.

Conclusions

The septal epithelium in region (ii) of the rat nasal cavity is proposed as a suitable test surface for the assessment of the histological effects of absorption enhancer solutions, with a 20 μl dose volume delivered to one side of the cavity only. Good contact with test solution may be assumed, the epithelium is consistent over a reasonably large area and the undosed side may be used as the control tissue. Both sides of the septum in one section may be observed in one field of view under the light microscope enabling easy qualitative assessment.

The histological effects of formulation factors other than the inclusion of absorption enhancers, could also be assessed using this model. Changes of drug dose, solution pH and osmolarity which have been investigated to improve the nasal absorption of some protein and peptide drugs (Chien, 1985; Ohwaki et al., 1985) may also have important effects on nasal epithelium integrity (Ohwaki et al., 1987). These effects, together with those due to different enhancer systems, will have implications regarding potential acute and chronic nasal dosage regimens, but may also provide further information as to the mechanisms of intranasal drug absorption and action of enhancers.

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