Pharmaceutical Research, Vol. 7, No. 5, 1990

Research Article

The Effects of Permeation Enhancers on the Surface Morphology of the Rat Nasal Mucosa: A Scanning Electron Microscopy Study

Richard D. Ennis,[1] Lucy Borden,[1] and William A. Lee[1,2]

Received June 26, 1989; accepted November 26, 1989

A rat model has been developed to compare relative morphological changes in the nasal mucosa after exposure to potential membrane permeation enhancers. Scanning electron microscopy was used to characterize gross structural and specific cellular changes following exposure. Micrographs of the rat nasal mucosa were scored in four categories: (1) mucosal surface integrity, (2) ciliary morphology, (3) mucus/extracellular debris, and (4) presence of red blood cells. The order of increasing morphological damage resulting from a 5-min exposure to each surfactant was 0.5% Solulan C-24 $\simeq$ 0.5% Solulan C-24/0.5% sodium tauro-24,25-dihydrofusidate (STDHF) < 0.5% STDHF < 1.0% STDHF $\ll$ 1.0% Laureth-9 < 1.0% sodium taurodeoxycholate $\simeq$ 1.0% sodium deoxycholate. The changes observed in the mucosal morphology after exposure to the various surfactants are in general agreement with data in the literature. This model is able to compare rapidly the relative morphological effects on the mucosal membrane of different nasal formulations.

KEY WORDS: permeation enhancer; scanning electron microscopy; nasal mucosa; toxicity; fusidic acid; morphology; bile salt; surfactant; nasal delivery.

INTRODUCTION

The systemic delivery of protein and peptide therapeutics by nasal administration is currently an active area of research and development. Small peptides can be readily absorbed through the nasal mucosa, but larger molecules show little or no systemic absorption when delivered in simple aqueous formulations (1–3). This poor bioavailability for larger peptides and proteins can be improved by the use of permeation enhancers. A large number of surfactants, including bile salts, nonionic polyoxyalkylenes, zwitterionics, fusidic acid derivatives, fatty acids, and phospholipids have been used to enhance the nasal absorption of macromolecules with varying degrees of success (4–6).

The mechanism by which different surfactant types increase the permeability across the nasal mucosa is not generally known (7). Given their structural dissimilarity, it is unlikely that identical mechanisms are operable for each permeation enhancer. It has been postulated that the enhancing effect of the bile salts as well as other surfactant types is due to their ability to erode epithelial cells and thus permanently alter the structural integrity of the mucosal membrane (8,9). The membrane damaging effects of long-chain anionic surfactants and nonionic polyoxyethylenes are well known (10). In addition, the damaging effects of bile salts on intestinal membranes (11), erythrocytes (12), hepatocytes (13), and liposomal membranes (14) have been extensively studied. Because of the potential for structural damage to the mucosal membrane, the safety of any surfactant being considered for use as a nasal permeation enhancer must be carefully evaluated. The acceptability of a permeation enhancer is dependent not only on its ability to enhance absorption, but also on its overall safety profile with regard to both local and systemic effects.

In order to compare the potential of different permeation enhancers to cause structural changes in the nasal mucosa, we have developed an *in vivo* rat exposure model and used scanning electron microscopy (SEM) to evaluate the morphological changes in the nasal mucosal membrane. Bile salts, nonionic surfactants, sodium tauro-24,25-dihydrofusidate (STDHF), and a mixed surfactant system containing STDHF and a nonionic surfactant was examined as a function of concentration and exposure time. Except for Solulan C-24 (15), these molecules were chosen because they have all been used clinically to enhance the intranasal absorption of protein and peptide therapeutics (5,16–18). A graded, quantitative evaluation of the induced morphological changes in the mucosal membrane yielded data consistent with related studies published in the literature.

MATERIALS AND METHODS

Materials

Sodium tauro-24,25-dihydrofusidate (STDHF) was obtained from Leo Pharmaceutical Products Ltd. (Ballerup,

[1] Drug Delivery Department, California Biotechnology Inc., 2450 Bayshore Parkway, Mountain View, California 94043.
[2] To whom correspondence should be addressed.

0724-8741/90/0500-0468$06.00/0

Denmark). Sodium taurodeoxycholate (STDC) was from Calbiochem (La Jolla, Calif.). Sodium deoxycholate (SDC) was from BDH Chemicals Ltd (Poole, England) and sodium glycocholate (SGC) was from Sigma Chemical Co. (St. Louis, Mo.). Polyoxyethylene-9-lauryl ether (Laureth-9) was obtained from PVO International Inc. (Boonton, N.J.) and polyoxyethylene-24-cholesterol ether (Solulan C-24) was from Amerchol Corporation (Edison, N.J.). All of the surfactants tested were used as supplied by the manufacturers. Glutaraldehyde (EM grade) and osmium tetroxide (crystalline) were obtained from Ted Pella, Inc. (Tustin, Calif.). All water used was deionized and pyrogen free. All other reagents were purchased from standard suppliers and used without further purification.

In Vivo Exposure

All surfactant solutions were prepared in 20 m*M* sodium phosphate buffer, pH 7.4 ($HPO_4^=$), with the exception of SDC, which was formulated at pH 8.5 to prevent gel formation (19–21). Enhancers were formulated at 0.5 and 1.0% (w/v).

Male Sprague–Dawley rats (Bantin and Kingman, Fremont, Calif.), weighing between 240 and 300 g each, were injected intraperitoneally with sodium pentobarbitol (65 mg/kg) to induce anesthesia. The surgical procedure for administration of the solutions to the nasal cavity was a modification of a procedure described in the literature (22,23). Polyethylene tubing, PE-200 and PE-90, was inserted into the trachea and nasopharynx, respectively. The PE-90 tubing was sealed into place at the nasopharyngeal opening using a cyanoacrylate adhesive. Animals were placed ventral side up on heating pads to maintain normal body temperatures (Fig. 1).

One milliliter of solution was infused into the nasal cavity over a period of 30 sec, resulting in the flooding of the cavity and the flow of excess solution out the nares. The solution which did not flow out the nares was left within the nasal cavity for the designated exposure time [volume of the rat nasal cavity, ≈0.4 ml (24)]. The cannulation of the nasopharyngeal opening prevented ciliary clearance of the surfactant solutions.

The exposure times necessary to cause changes in the mucosa morphology were determined in preliminary experiments by comparing mucosal tissue exposed to 1.0% solutions of STDHF, to 1.0% Laureth-9, and to 1.0% SGC with control treatments. Control treatment consisted of exposure to 20 m*M* $HPO_4^=$, pH 7.4. Based on these experiments, exposure times of 5, 10, or 15 min were selected. Exposures were terminated by perfusing through a solution of 0.15 *M* NaCl for 5 min at a flow rate of 1.5 ml/min. This was followed by a 5-min perfusion (1.5 ml/min) with 2% glutaraldehyde (w/v) in 100 m*M* sodium phosphate buffer (pH 7.4). The nasoturbinates, maxilloturbinates, and lateral wall from both the left and the right sides of the nasal cavity were excised in a single piece with the underlying cartilage attached (Fig. 2).

Tissue Preparation

Excised tissue was immediately immersed in 2% glutaraldehyde at pH 7.4 (100 m*M* phosphate) and fixed overnight at 4°C. Postfixation was carried out in 1% OsO_4 (w/v) for 1 hr at room temperature. Tissue was then washed with phosphate buffer, dehydrated in EtOH, critical point dried, and sputter coated with platinum to a thickness of 25 nm.

Micrography

The nasal mucosa tissue was examined in a Hitachi ISI DS-130 electron microscope at either 5 or 10 kV. Photomicrographs were taken from standardized areas on the outer wall of the naso- and maxilloturbinates approximately 100 to 200 μm from the tips of the turbinates (Fig. 2). A minimum of one micrograph was taken from the anterior, medial, and posterior regions of either the naso- or the maxilloturbinates. Each standardized area was photographed using dual magnification (~1000 and ~2000×). Images were recorded on Polaroid P/N 55 film.

Tissue Evaluation

For each exposure condition, a minimum of two rats was treated, and both the left and the right turbinates were removed. After surgical removal of the tissue, samples were coded and subsequent tissue preparation and microscopy

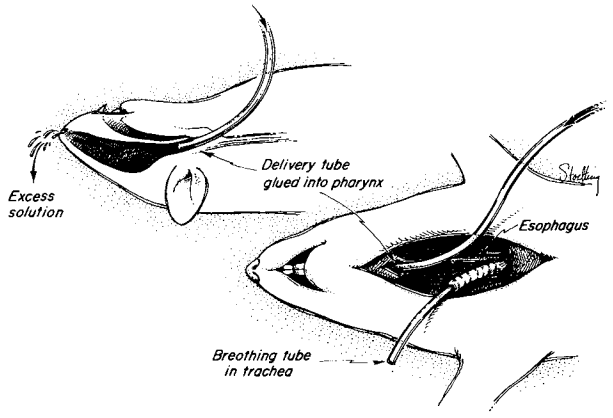

Fig. 1. *In vivo* rat exposure model. The entire mucosal surface is exposed to surfactant solution and clearance is prevented by cannulation of the pharyngeal opening.

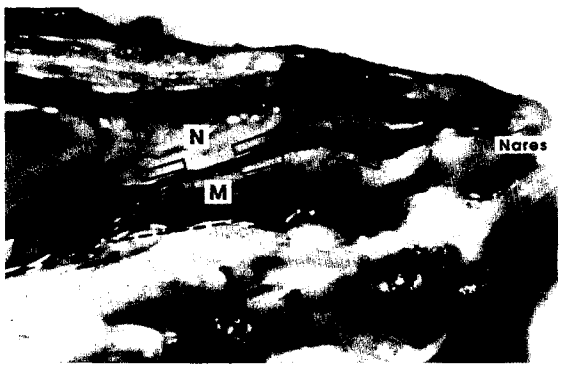

Fig. 2. The lateral wall of the left nasal cavity showing the nasoturbinate (N) and maxilloturbinate (M). The dashed line outlines the tissue which was surgically removed. Photomicrographs were taken from the anterior, medial, and posterior regions which are designated by the rectangles.

were carried out in a blind fashion. A minimum of 3 photomicrographs was taken from each tissue sample, yielding at least 12 photomicrographs per treatment. Tissue damage during surgical excision or tissue preparation reduced the number of micrographs obtained for some treatments to less than 12.

All photomicrographs were grouped according to anterior, medial, and posterior regions of origin and randomized in each group. Each photomicrograph was scored from 1 to 5 in the four categories shown in Table I. The micrographs were evaluated by eight individuals familiar with mucosal ultrastructure. A mean score in each of the four categories was calculated for the individual micrographs. Micrographs were then unblinded and grouped according to treatment. The final score for each treatment was the mean of the mean scores for each of the photomicrographs in a given treatment group. The number of micrographs evaluated for each condition is shown in Table II. The population distribution of the individual means was tested for normality using the Wilk–Shapiro test. The F test was used to test for equal variances between any two treatments. Significant differences between groups were assessed using the pooled-variance t test when the F test showed equal variances or the unpooled-variance t test when the F test showed unequal variances.

RESULTS

Representative scanning electron micrographs for all of the permeation enhancers tested are shown in Figs. 3–13. At 1–2 K magnification the micrographs very clearly show changes in membrane morphology with different formulations and varying exposure times. The morphological changes in the presence of the various surfactants range from no discernible change from the control to severe membrane erosion. Other changes include ciliary fusion, cell separation, mucus secretion, and cellular distortion. The large cracks visible in some micrographs are due to differential shrinkage between the soft tissue and the cartilage during the dehydration and critical-point drying process and were ignored for scoring purposes.

The results of the subjective evaluation for each exposure condition in the four categories listed in Table I are summarized in Table II. The scores in categories 1–3 exhibit almost identical trends for a given solution and exposure time, whereas the presence of red blood cells (category 4) shows no correlation to the other three. The greatest degree of differentiation ($P < 0.05$) in the treatment scores is found in categories 1 and 2, the overall appraisal of membrane integrity and ciliary morphology. Membrane integrity scores (category 1) after a 5-min exposure to each of the solutions tested are shown in Fig. 14. The scores increased (representing increasing membrane damage) after a 5-min exposure in the following order ($P < 0.05$): $HPO_4^{=} \simeq 0.5\%$ Solulan C-24 $\simeq 0.5\%$ Solulan C-24/0.5% STDHF $< 0.5\%$ STDHF $< 1.0\%$ STDHF $\simeq 1.0\%$ SGC $\ll 1.0\%$ Laureth-9 $< 1.0\%$ STDC $\simeq 1.0\%$ SDC. The scores for SGC after a 5-min exposure are anomalously high when compared to the results observed at the 10- and 15-min exposure times (the combined scores for the two 5-min exposure treatments were also not normally distributed). Due to the gross alterations in the mucosal membrane observed after a 5-min exposure to 1% Laureth-9, 1% STDC, and 1% SDC, no longer exposure times were examined. The micrographs in Figs. 11–13 depict the severe erosion for these three surfactants after a 5-min exposure time. Figures 12 and 13 show mucosal membranes which have been wholly or partially removed, leaving basal cells and the lamina propria exposed.

Table I. Categories Used to Evaluate the Scanning Electron Micrographs

I	Mucosal surface integrity (1 = normal, 5 = unrecognizable)
II	Ciliary morphology[a] (1 = normal, 5 = gross deformation)
III	Mucus/extracellular debris (1 = little, 5 = abundant)
IV	Red blood cells (1 = none, 5 = many)

[a] Ciliary morphology was evaluated only for those micrographs representing the middle and posterior regions (Fig. 2) due to the sparse population of ciliated cells in the anterior region.

The effects of exposure time were investigated for phosphate buffer, 0.5 and 1.0% STDHF, and 1.0% SGC. The membrane integrity scores (category 1) at the different exposure times are illustrated in Fig. 15. Exposure to the phosphate buffer solution for 5, 10, or 15 min did not result in significantly different scores ($P < 0.05$). In the case of 0.5% STDHF, a 5-min exposure scored lower than the 10- or 15-min exposures in category 1 ($P < 0.05$). For the 1.0% STDHF solution, there were no significant differences in the scores at any of the exposure times for categories 1–3 ($P < 0.05$). For the 1.0% SGC solution, the scores in categories 1–3 decreased between 5 and 10 min, however, the significance of the higher score at 5 min is questionable.

The effects of STDHF concentration on the nasal mucosa were compared at 0.5 and 1.0% and in combination with the nonionic surfactant Solulan C-24. The 5-min exposure to the 0.5% solution of STDHF alone resulted in a lower membrane integrity score than the 1.0% STDHF solution, whereas there were no differences ($P < 0.05$) in any of the scores for the 0.5 and 1.0% concentration after a 10- or 15-min exposure time. The 5-min exposure to 0.5% Solulan C-24 or the combination of 0.5% Solulan C-24/0.5% STDHF resulted in scores for categories 1–3 which were not different ($P < 0.05$) from the scores for a 5-min exposure to phosphate buffer alone.

In addition to the standardized pictures taken in the maxillary turbinate area, other regions of the nasal cavity, including the septum, dorsal meatus surfaces, and nasopharynx, were examined for selected treatments. The exposure results were qualitatively similar.

DISCUSSION

The objective of this study was to develop a relevant and sensitive *in vivo* model to evaluate the induced changes or damage to the nasal mucosal membrane as a consequence of exposure to different permeation enhancers. The rat was chosen as the animal model because of the histological data available on the rat nasal cavity (24) and the widespread utilization of the rat in nasal drug delivery studies. Scanning electron microscopy (SEM), which has been used previously to characterize the normal ultrastructure of the rat nasal respiratory epithelium (25), proved to be an excellent tech-

Table II. Mean Scores (±SE) for Each Treatment

Treatment	Time (min)	Category[a] 1	2	3	4	N^b	R^c
STDHF (0.5%, w/v)	5	2.10 ± 0.16	2.21 ± 0.19	1.88 ± 0.11	1.86 ± 0.20	24	1.0–3.9
"	10	2.62 ± 0.15	2.29 ± 0.18	2.13 ± 0.11	1.97 ± 0.10	26	1.1–4.4
"	15	2.62 ± 0.27	2.77 ± 0.27	2.12 ± 0.19	1.68 ± 0.12	12	1.3–4.3
STDHF (1.0%, w/v)	5	2.77 ± 0.23	2.98 ± 0.46	2.87 ± 0.23	2.04 ± 0.28	13	1.3–4.0
"	10	2.76 ± 0.22	3.31 ± 0.66	2.71 ± 0.37	1.67 ± 0.18	6	2.3–3.5
"	15	3.06 ± 0.25	3.10 ± 0.32	3.12 ± 0.27	2.30 ± 0.31	9	1.7–4.0
Laureth-9 (1%, w/v)	5	3.88 ± 0.29	4.21 ± 0.41	3.51 ± 0.26	2.61 ± 0.31	11	2.0–4.7
STDC (1%, w/v)	5	4.74 ± 0.07	4.85 ± 0.07	4.59 ± 0.10	1.87 ± 0.21	12	4.1–5.0
SDC (1%, w/v)	5	4.91 ± 0.05	5.00 ± 0.00	4.55 ± 0.14	2.03 ± 0.43	6	4.7–5.0
SGC (1%, w/v)	5	2.85 ± 0.35	2.82 ± 0.47	2.75 ± 0.32	2.09 ± 0.28	18	1.0–5.0
"	10	1.79 ± 0.24	1.67 ± 0.19	1.49 ± 0.17	1.50 ± 0.23	9	1.0–3.2
"	15	1.55 ± 0.16	1.45 ± 0.09	1.60 ± 0.17	1.32 ± 0.10	8	1.1–2.5
Buffer control	5	1.43 ± 0.12	1.38 ± 0.15	1.41 ± 0.16	2.01 ± 0.33	9	1.0–2.1
"	10	1.34 ± 0.06	1.57 ± 0.15	1.29 ± 0.07	1.30 ± 0.10	11	1.0–1.7
"	15	1.47 ± 0.08	1.31 ± 0.08	1.36 ± 0.08	1.51 ± 0.15	21	1.0–2.3
Solulan C-24 (0.5%, w/v)	5	1.61 ± 0.11	1.82 ± 0.15	1.42 ± 0.07	1.72 ± 0.20	12	1.1–2.4
Solulan C-24/STDHF (0.5%/0.5%, w/v)	5	1.55 ± 0.10	1.63 ± 0.10	1.46 ± 0.10	2.07 ± 0.28	12	1.1–2.1

[a] Scores for each category represent the mean of the mean scores for each micrograph after evaluation by eight individuals.
[b] Total number of micrographs scored for given treatment. Each micrograph was scored by eight individuals.
[c] Range of the category 1 mean micrograph scores for each treatment.

nique for evaluating gross structural alterations and specific cellular changes induced by exposure to different surfactant solutions. To ensure complete exposure of the rat nasal mucosal membrane to the surfactant solution, 1 ml of the solution was pushed through the nasopharyngeal cavity, resulting in the flow of excess solution from both frontal nares. Flooding the nasal cavity with solution is necessary since lesser volumes may not uniformly expose the mucosal tissue, potentially leading to erroneous comparisons. Furthermore, the cannulation of the nasopharyngeal opening prevents the rat from clearing the solution through the esophagus, thereby ensuring constant and complete exposure of the entire mucosal surface. The maxilloturbinate and nasoturbinate were the primary sites chosen for evaluation due to their large relative surface area, distribution of cell types, and a location in the nasal cavity which ensures their exposure to inspired solutions (23). It is also postulated that the turbinates, which comprise the majority of the nasal re-

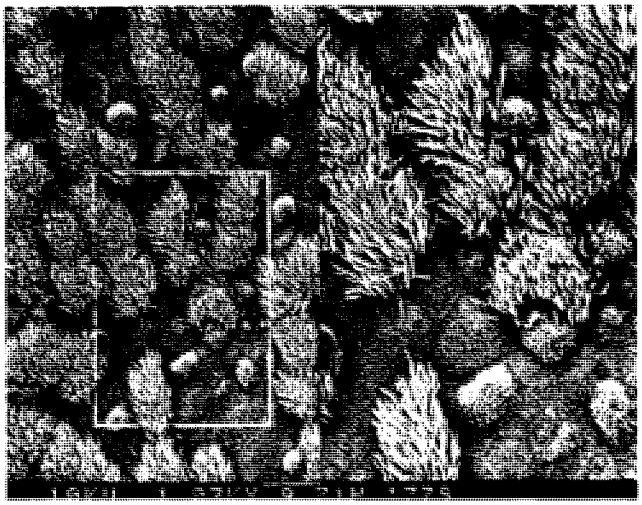

Fig. 3. Micrograph showing the medial region of the turbinate after a 15-min exposure to 20 m*M* phosphate buffer (control). Mean category 1 scores: micrograph shown = 1.4; overall treatment score = 1.5.

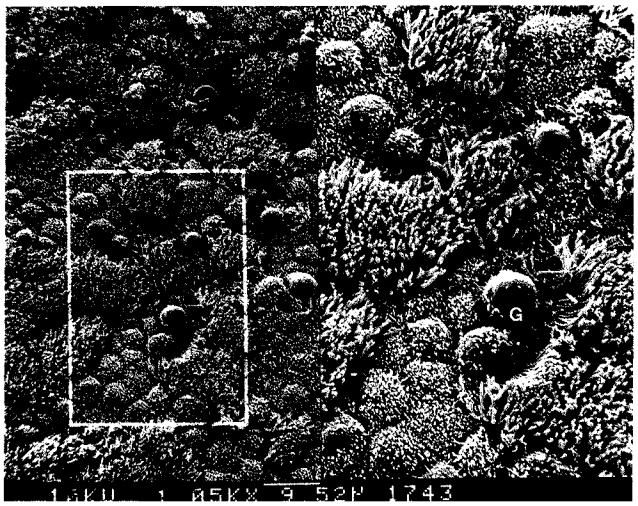

Fig. 4. Micrograph showing the anterior region of the turbinate after a 15-min exposure to 0.5% Solulan C-24. Certain goblet cells (G) appear rounded. Mean category 1 scores: micrograph shown = 1.7; overall treatment score = 1.6.

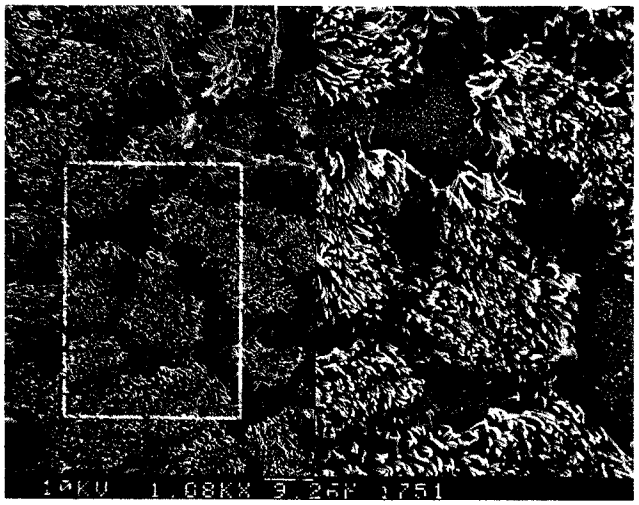

Fig. 5. Micrograph showing the medial region of the turbinate after a 15-min exposure to 0.5% Solulan C-24/0.5% STDHF mixed micelle solution. Several goblet cells are rounded, an occurrence which precedes mucous secretion. Mean category 1 score: micrograph shown = 1.6; overall treatment score = 1.5.

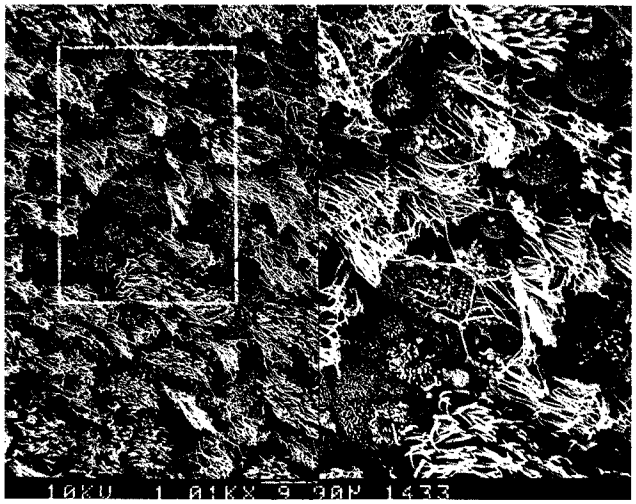

Fig. 7. Micrograph from the posterior region of the turbinate after a 5-min exposure to 0.5% STDHF. Mean category 1 scores: micrograph shown = 2.7; overall treatment score = 2.1.

spiratory tissue in man, are the primary sites for systemic absorption of nasally administered drugs (26).

The surfactants can be divided into three groups based on their scores in categories 1–3, with 1.0% STDC, 1.0% SDC, and 1.0% Laureth-9 scoring the highest (4–5), STDHF (0.5% and 1.0%) scoring intermediate (2–3), and 1.0% SGC (excluding the 5-min time point), $HPO_4^=$, 0.5% Solulan C-24, and the 0.5% STDHF/0.5% Solulan C-24 combination scoring the lowest (1–2). Qualitatively, there were no differences among the control, a 20 m*M* $HPO_4^=$ solution, and published SEM micrographs of normal rat mucosa (25). The scores in categories 1 and 2, the evaluation of overall membrane integrity and the ciliary morphology, show the greatest degree of differentiation between the surfactant solutions tested. However, since cilia are found primarily in the middle and the posterior portions of the turbinates and are almost absent in the anterior regions, category 1 is the most useful for evaluating the overall effects of surfactants on the mucosa. The lack of correlation between categories 1–3 and the abundance of red blood cells (category 4) is likely due to the accumulation of RBCs during surgical removal of the turbinates and does not reflect surfactant specific effects.

Surprisingly the duration of the exposure did not result in large differences in the scores. Only in the case of 0.5% STDHF was there a statistically significant ($P < 0.05$) lower score in category 1 for the 5-min exposure relative to the 10-min exposure. There were no significant differences between the 10- and the 15-min exposure scores for the 0.5% STDHF, 1.0% STDHF, 1.0% SGC, or phosphate buffer treatments. The lack of additional changes to the mucosal surface (except in the case of 0.5% STDHF) at the longer exposure times can be accounted for either by a rapid ab-

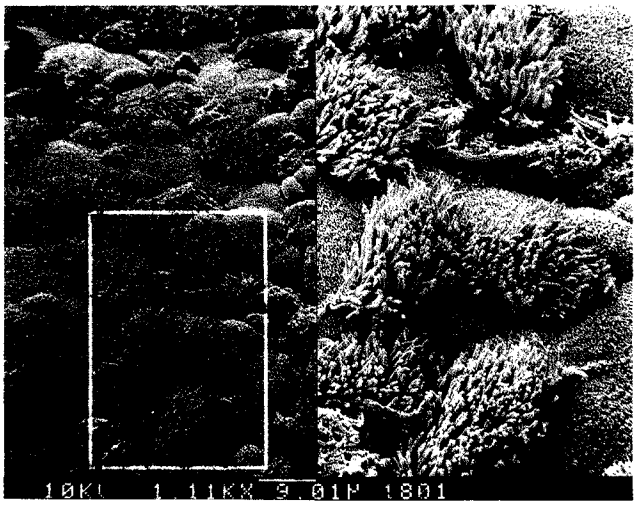

Fig. 6. Micrograph showing the medial region of the turbinate after exposure to 1.0% SGC for 10 min. Mean category 1 scores: micrograph shown = 1.6; overall treatment score = 1.8.

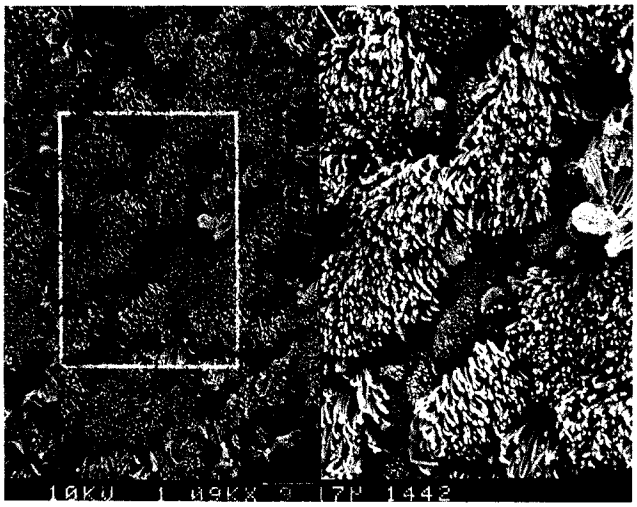

Fig. 8. Micrograph from the anterior region of the turbinate after a 10-min exposure to 0.5% STDHF. Goblet cells and ciliated cells are visible. Mean category 1 scores: micrograph shown = 2.6; overall treatment score = 2.6.

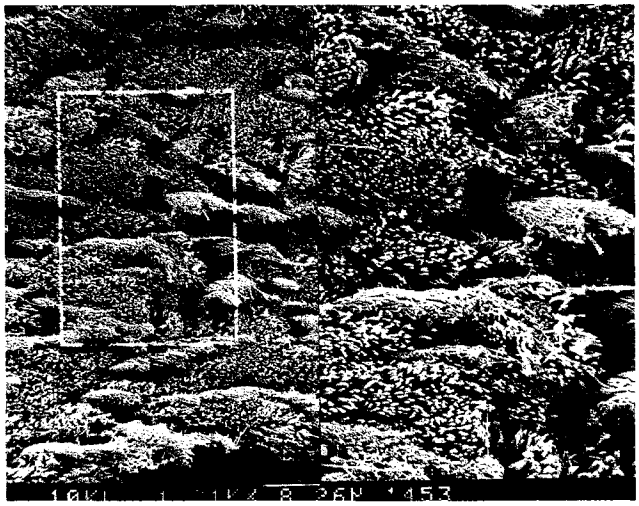

Fig. 9. Micrograph showing the medial region of the turbinate after a 5-min exposure to 1.0% STDHF. Some cilia appear fused or may be covered with mucous secretions. Mean category 1 scores: micrograph shown = 2.1; overall treatment score = 2.8.

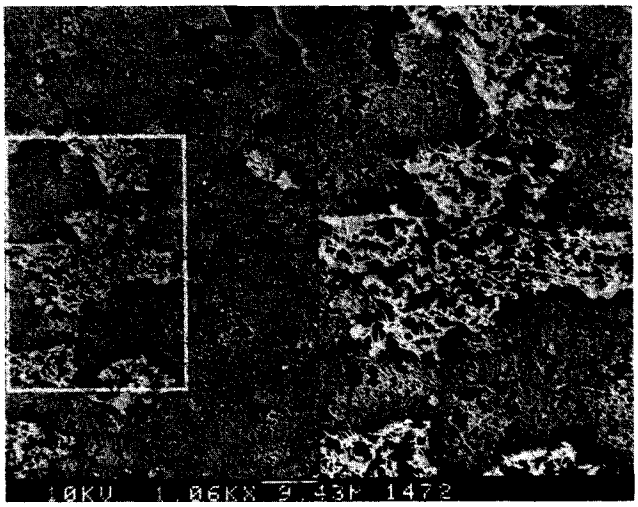

Fig. 11. Micrograph showing the anterior region of the turbinate after a 5-min exposure to 1.0% Laureth-9. Cells appear to be depleted of membrane components and are highly porous. The cilia are thin and fused. Mean category 1 scores: micrograph shown = 3.7; overall treatment score = 3.9.

sorption of the solution from the nasal cavity, thereby decreasing the effective concentration of surfactant, or by assuming that the process(es) which results in the observed morphological changes is essentially complete within the first 5 min of exposure.

The relative morphological effects on the rat mucosa after exposure to the three bile salts examined in this study (SGC, SDC, and STDC) are in general agreement with published data which suggest that the cytotoxicity is inversely proportional to the degree of hydroxylation on the steroid nucleus (13,14). In a separate study utilizing SEM to compare the effects of 1.0% SGC to 1.0% Laureth-9, Hirai *et al.* found that the slight changes in the mucosal morphology after exposure to SGC were completely reversible within 24 hr, but the more severe changes observed with Laureth-9 were only partially recoverable (7). We have not addressed the potential for mucosal recovery in our study.

It has been frequently observed that an increase in drug permeability in the presence of different surfactants is invariably accompanied by structural changes in the mucosal membranes (8,10). Hirai *et al.* have compared the effects on both bioavailability and membrane damage for a series of nonionic surfactants, sodium lauryl sulfate, and trihydroxy bile salts (7). In general for the nonionic surfactants, a good correlation existed between the absorption promoting effects of the surfactant and the lysis of red blood cells. In contrast, some bile salts showed reduced lytic capacity but equal absorption promoting effects. This suggests that for certain surfactants the mechanism for enhancing absorption is independent of the membrane damaging process. In a subsequent

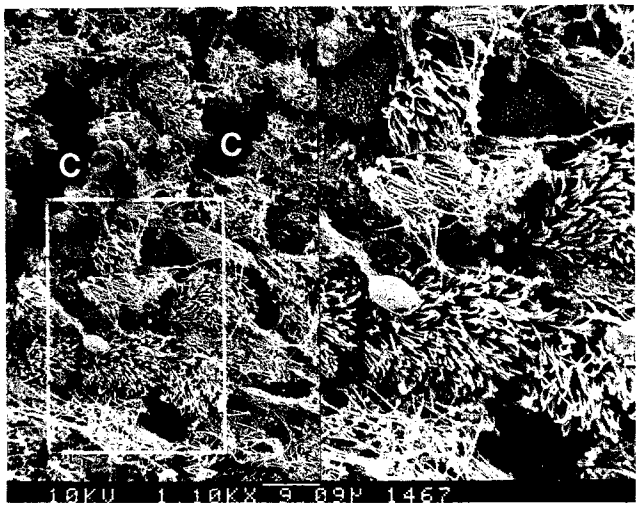

Fig. 10. Micrograph showing the medial region of the turbinate after exposure to 1% STDHF for 15 min. Large cracks (C) are visible which are due to differential shrinkage and swelling during tissue processing for SEM. Mean category 1 scores: micrograph shown = 3.4; overall treatment score = 3.1.

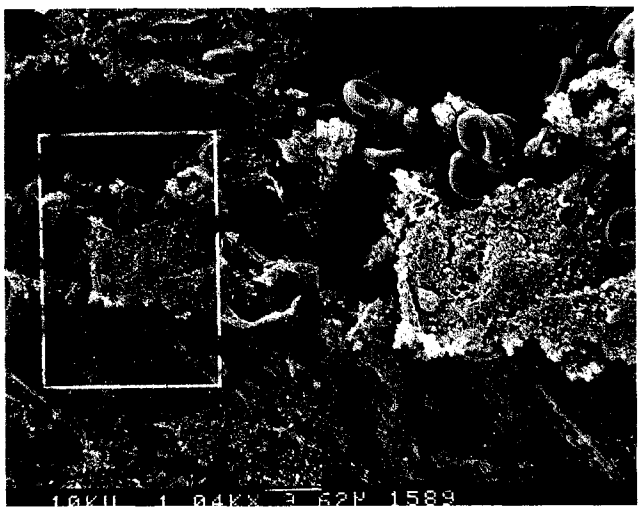

Fig. 12. Micrograph showing the anterior region of the turbinate after a 5-min exposure to 1.0% SDC. Loss of cellular identity and prevalence of extracellular debris are seen. Mean category 1 scores: micrograph shown = 4.9; overall treatment score = 4.9.

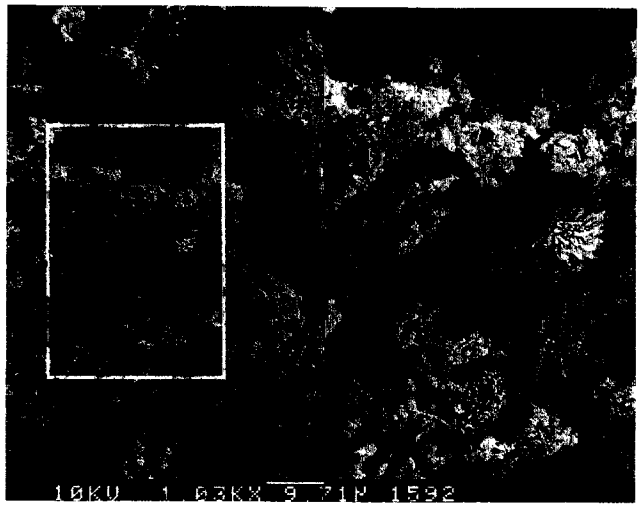

Fig. 13. Micrograph showing the anterior region of the turbinate after a 5-min exposure to 1.0% STDC. Surfactant exposure has severely eroded most cells from the epithelium. One loosely attached ciliated cell is still visible. Mean category 1 scores: micrograph shown = 4.7; overall treatment score = 4.7.

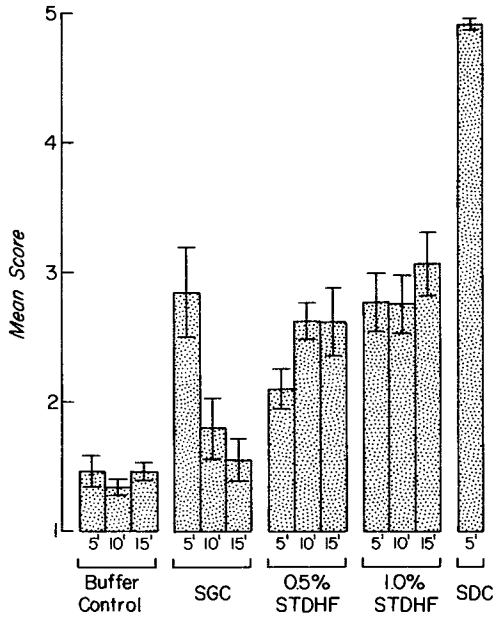

Fig. 15. The mean scores (±SE) for membrane integrity (category 1) for four treatments as a function of exposure time.

study, we report on the bioavailabilities of insulin and human growth hormone with each of the surfactants tested in this study (27).

The toxicological model developed in this study is useful for comparing the relative effects of different formulations on the morphology of the nasal mucosa. It has been possible to observe specific structural alterations caused by different surfactant classes. The relevance of the model to a clinical situation has not been established. Because of the model design, the exposure conditions are severe relative to what is encountered in a clinical situation, and therefore, the histological alterations observed probably do not represent those observed after single or chronic dosing in the clinic. This assumption is suggested by the results of a 90-day subchronic study in dogs, where STDHF formulations up to 10% were administered intranasally thrice daily and no histological changes relative to the control animals were observed (28). The usefulness of this model lies in its ability to visualize and compare the relative effects on a nasal mucosa membrane after exposure to different formulations.

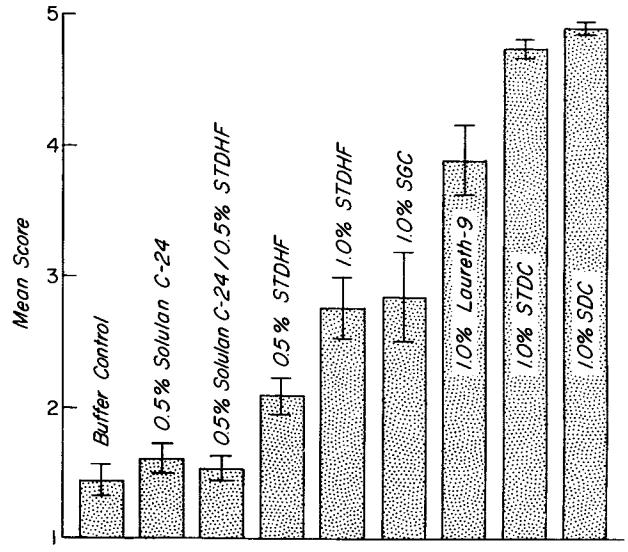

Fig. 14. The mean scores (±SE) for membrane integrity (category 1) after a 5-min exposure to each solution.

ACKNOWLEDGMENTS

We are grateful to the Drug Delivery Department for their careful evaluation of the scanning electron micrographs; the Electron Microscope Laboratory, University of California, Berkeley, for use of their facility; and to Eric Stoelting for his illustrations.

REFERENCES

1. C. McMartin, L. E. F. Hutchinson, R. Hyde, and G. E. Peters. *J. Pharm. Sci.* 76:535–540 (1987).
2. K. S. E. Su. *Pharm. Int.* 7:8–11 (1986).
3. W. A. Lee and J. P. Longenecker. *Biopharm. Manuf.* 1:30–37 (1988).
4. S. Hirai, T. Yashiki, and H. Mima. *Int J. Pharm.* 9:165–172 (1981).
5. G. S. Gordon, A. C. Moses, R. D. Silver, J. S. Flier, and M. C. Carey. *Proc. Natl. Acad. Sci. USA* 82:7419–7423 (1985).
6. A. L. Daugherty, H. D. Liggitt, J. G. McCabe, J. A. Moore, and J. S. Patton. *Int. J. Pharm.* 45:197–206 (1988).
7. S. Hirai, T. Yashiki, and H. Mima. *Int. J. Pharm.* 9:173–184 (1981). (See also Refs. 1, 3, and 6.)
8. S. J. Hersey and R. T. Jackson. *J. Pharm. Sci.* 76:876–879 (1987).
9. M. Gibaldi. *Fed. Proc.* 29:1343–1349 (1970).
10. D. Attwood and A. T. Florence. In *Surfactant Systems,* Chapman and Hall, New York, 1983, pp. 619–649.
11. J. W. Keeling, S. P. Lamabadusuriya, and J. T. Harries. *J. Pathol.* 118:157–165 (1976).
12. D. Billington and R. Coleman. *Biochim. Biophys. Acta* 509:33–47 (1978).
13. J. Schoelmerich, M. S. Becher, K. Schmidt, R. Schubert, B.

Kremer, S. Feldhaus, and W. Gerok. *Hepatology* 4:661–666 (1984).
14. R. Schubert, H. Jarone, J. Schoelmerich, and K. H. Schmidt. *Digestion* **28**:181–190 (1983).
15. M. Azria and T. Cavanak. UK Patent Application, GB 2 127 689 A (1984).
16. M. S. Nolte, C. Taboga, E. Salamon, L. Silviera, A. Moses, P. H. Forsham, and J. H. Karam. Proc. 47th Sci. Sess. Meet., American Diabetes Association (1987).
17. R. Salzman, J. E. Manson, G. T. Griffing, R. Kimmerle, N. Ruderman, A. McCall, E. I. Stoltz, C. Mulin, D. Small, J. Armstrong, and J. C. Melby. *N. Engl. J. Med.* **312**:1078–1084 (1985).
18. A. E. Pontiroli, M. Alberetto, A. S. Secchi, G. Dossi, I. Bosi, and G. Pozza. *Br. Med. J.* **284**:303–306 (1982).
19. A. Rich and D. M. Blow. *Nature* **182**:423–426 (1958).
20. D. M. Blow and A. Rich. *J. Am. Chem. Soc.* **82**:3566–3571 (1960).
21. H. Sobotka and N. Czeczowiczka. *J. Colloid Sci.* **13**:188 (1958).
22. H. Salem and E. Clemente. *Arch. Otolaryngol.* **96**:524–529 (1972).
23. S. Hirai, T. Yashika, T. Matsuzawa, and H. Mima. *Int. J. Pharm.* 7:317–325 (1981).
24. J. P. Schreider. In *Toxicology of the Nasal Passages,* C. S. Barrow (ed.), Hemisphere, Washington, DC, 1986, pp. 1–25.
25. J. A. Popp and J. T. Martin. *Am. J. Anat.* **169**:425–436 (1984).
26. G. D. Parr. *Pharm. Int.* 202–205 (1983).
27. W. A. Lee *et al.* In preparation.
28. J. P. Longenecker. *Internal Toxicity Reports,* California Biotechnology, Mountain View, CA, 1986.