Pharmaceutical Research, Vol. 7, No. 8, 1990

Research Article

The Molecular Weight Dependence of Nasal Absorption: The Effect of Absorption Enhancers

Maureen D. Donovan,[1-3] Gordon L. Flynn,[1] and Gordon L. Amidon[1]

Received November 13, 1989; accepted January 30, 1990

A series of polyethylene glycols (PEGs) ranging in molecular weight from near 600 to over 2000 daltons was used to study the effects of three absorption enhancers (sodium glycocholate, sodium lauryl sulfate, and polyoxyethylene 9 lauryl ether) on the molecular weight permeability profile of the nasal mucosa of the rat. Molecular weight–permeability properties were studied both by following changes in the excretion of the polyethylene glycols as a function of their molecular size and by examining the nasal mucosa for morphologic changes following exposure to the PEG/enhancer mixtures. Each absorption enhancer was found to affect the mucosa and its permeability in a unique manner. At a 1% concentration, sodium glycocholate only slightly affects tissue morphology and does not significantly alter the molecular weight permeability profile of the mucosa. In contrast, 1% sodium lauryl sulfate causes severe alteration of the mucosa and also greatly increases the absorption of both the PEG 600 and the PEG 2000 oligomers. Polyoxyethylene 9 lauryl ether was found to exert its action in a concentration-dependent manner. At a concentration of 0.1%, few changes were seen in either mucosal integrity or permeability. At a 1% concentration, however, a significant alteration in the structure of the mucosal tissues as well as a profound increase in the permeability of the mucosa to the PEGs was observed. Correlation of mucosal integrity with the effectiveness of an enhancer indicates that some of these compounds appear to be acting by altering the structure of the mucosa. Others, which appear to exert a less damaging effect on the mucosal cells themselves, achieve their greatest absorption enhancement when changes in cell-to-cell adhesion in the mucosa are observed. These results indicate that the paracellular routes may play an important role in large molecule absorption through the nasal mucosa.

KEY WORDS: nasal absorption; absorption enhancer; adjuvant; polyethylene glycol; sodium glycocholate; sodium lauryl sulfate; polyoxyethylene 9 lauryl ether.

INTRODUCTION

The therapeutic potential of synthetic, biologically active peptides has increased the need for acceptable nonparenteral, nonoral routes of drug delivery. The nasal mucosa has been shown to be a site from which some peptide compounds are absorbed systemically. The nasal mucosa is composed primarily of a pseudostratified columnar epithelium which, because of the presence of microvilli, has a large surface area available for absorption. The tissue is highly vascularized and provides ready access to the circulatory system while avoiding first-pass metabolism by the liver. Systemic absorption from the nasal cavity has been reported for compounds ranging from traditional oral medications such as scopolamine (1), hydralazine (2), and propranolol (3), to large polypeptides which are otherwise available only in parenteral dosage forms, such as insulin (4). However, many higher molecular weight compounds show significant nasal absorption only when an absorption enhancer is included in the formulation. Since a marked reduction in the bioavailability of compounds from the nasal cavity occurs with increasing molecular weight (5–8), the improved absorption of large compounds in the presence of the enhancers suggests that the adjuvants can alter the intrinsic molecular weight permeability profile of the mucosa.

Compounds reported to act as nasal absorption adjuvants include anionic and nonionic surfactants (9), bile acids and their salts (10), fusidic acid derivatives (11), the topical antibiotic, bacitracin (12), medium-chain fatty acid salts (13), and combinations of fatty acids and bile salts (14). In this study, three absorption adjuvants, sodium glycocholate, polyoxyethylene 9 lauryl ether, and sodium lauryl sulfate, each an effective absorption enhancer for intranasal insulin (9), have been investigated. The marker compounds polyethylene glycol (PEG) 600, 1000, and 2000 were used to evaluate the adjuvant induced changes in the molecular weight permeability profile of the nasal mucosa (5). Mucosal samples were also taken from the rat nasal cavity following the *in situ* absorption experiments and were examined for histological changes.

[1] College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109.

[2] Present address: College of Pharmacy, University of Iowa, Iowa City, Iowa 52242.

[3] To whom correspondence should be addressed at The University of Iowa, College of Pharmacy, Iowa City, Iowa 52242.

0724-8741/90/0800-0808$06.00/0

MATERIALS AND METHODS

Solution Preparation

All PEG/adjuvant solutions consisted of 25% (w/v) of either PEG 600, 1000, or 2000 (Sigma Chemical Co., St. Louis, MO, or BASF Corp., Parsippany, NJ) with 1% (w/v) sodium glycocholate, sodium lauryl sulfate, polyoxyethylene 9 lauryl ether, or 0.1% polyoxyethylene 9 lauryl ether (Sigma Chemical Co., St. Louis, MO). All compounds were used as obtained. All mixtures were brought to volume with 0.9% sodium chloride.

Animal Studies

Male, Sprague–Dawley rats weighing from 250 to 350 g (9 to 13 weeks old) were used to measure the intranasal absorption of PEG 600, 1000, or 2000 in the presence of selected absorption adjuvants. At least three rats were used for each PEG/adjuvant combination. All animal procedures were approved by the University of Michigan Committee for Use and Care of Laboratory Animals.

The rats were anesthetized with an intramuscular injection of urethane [50% (w/v), 1.5 g/kg] (Sigma Chemical Co., St. Louis, MO). A warming blanket was used to maintain normal animal body temperature. The nasal cavity of each rat was isolated from the rest of its respiratory and gastrointestinal tract to eliminate loss of the instilled dose during the experiment. The isolation procedure was a modification of the technique originated by Hussain *et al.* (15) and has been described previously (5). A 50-μl volume of the test PEG/adjuvant solution was placed into each nostril using a piece of PE-10 tubing attached to a 0.5-ml syringe (Becton-Dickinson, Rutherford, NJ).

The absorption of PEG was measured by following the urinary excretion of the polymer over the 6-hr interval following dosing. Adequate urine output was ensured by the intravenous administration of 0.5 ml of heparinized (10 U/ml) normal saline every 30 min throughout the experiment through a femoral artery cannula made from PE-50 tubing. In order to collect the entire volume of urine excreted, the bladder of each rat was cannulated with a piece of polyethylene tubing (PE-100) using a purse string suture. The urine was collected in borosilicate glass test tubes and stored at −20°C until undergoing HPLC analysis for the presence of PEG.

Histology

Histological samples were prepared from tissues of sacrificed animals which had received either PEG 600 or 2000/adjuvant test solutions. The animals were sacrificed with an iv bolus overdose of anesthetic following the 6-hr urine collection period. The tissues of the entire nasal cavity were fixed by immediately thereafter perfusing a 500-ml volume of 10% buffered formalin (Fisher Scientific, Fair Lawn, NJ) through the rat's circulatory system via the carotid arteries. The solution was pumped at a rate of 4 ml/min using a Harvard infusion pump (Model 975, Harvard Apparatus, South Natick, MA). The tissues of the nasal cavity were then decalcified by placing them in a bath of 25% formic acid with 7.5% sodium citrate for 3 to 4 days. The wash solution was changed every 8 to 12 hr until no further dissolved calcium could be detected. The presence of calcium was determined by the appearance of the insoluble salt, calcium oxalate, formed upon adding an aliquot of wash solution to a solution of 5% sodium oxalate at a 5-to-1 proportion. The formic acid present in the tissues was neutralized and removed by placing the tissue sections in a bath of 5% sodium sulfate for 12 hr followed by rinsing them in running cold water for 24 hr.

Approximately 0.5-cm segments of tissue from the turbinate region of the main nasal cavity were excised and dehydrated in ethanol. The blocks were embedded in paraffin and 7-μm-thick samples were sectioned for microscopic examination. Three consecutive tissue sections were mounted on a single glass slide and stained with hematoxylin and eosin. The tissue morphology was evaluated using light microscopy.

Analytical Procedure

The polyethylene glycols were extracted and analyzed via HPLC using refractive index detection (5). The addition and subsequent excretion of the test absorption adjuvants did not interfere with the ability to quantitate the PEG oligomers.

RESULTS

Absorption Studies

It can be seen from Figs. 1–4 that each of the adjuvants studied affects the PEG molecular weight permeability profile in a unique, and sometimes concentration dependent, manner. The excretion values in each figure have been normalized to the known mean excretion of each PEG oligomer following intravenous administration (5).

Figure 1 shows the effect of 1% sodium glycocholate on PEG absorption. This bile salt causes virtually no absorption enhancement of PEG throughout the molecular weight range studied. Sodium lauryl sulfate, an anionic surfactant, has a more dramatic adjuvant effect (Fig. 2). In this case, the absorption of the oligomers of PEG 600 is increased approximately 2-fold and that of the oligomers of PEG 2000 approximately 10-fold. The degree of absorption of PEG 1000 in the presence of 1% sodium laurel sulfate does not appear to differ from the intrinsic PEG absorption. Selective molecular weight effects are also seen with 0.1% polyoxyethylene 9 lauryl ether. Absorption of the oligomers of PEG 600 is significantly improved, while the absorption of PEG 1000 and 2000 shows no enhancement. At a 1% concentration, the absorption of all of the oligomers is substantially increased and the molecular weight dependency of their absorption is considerably reduced.

Histology

Representative photomicrographs of the nasal epithelial samples are shown in Figs. 5–11b. Each micrograph shows the mucosa covering the scroll of a turbinate under 250× magnification (magnifications altered by photographic reduction). Table I summarizes the effect of each PEG/adjuvant solution on the morphology of the nasal mucosa. The grading criteria used are described in footnote *a*.

Figure 5 is a micrograph from a mucosal sample ex-

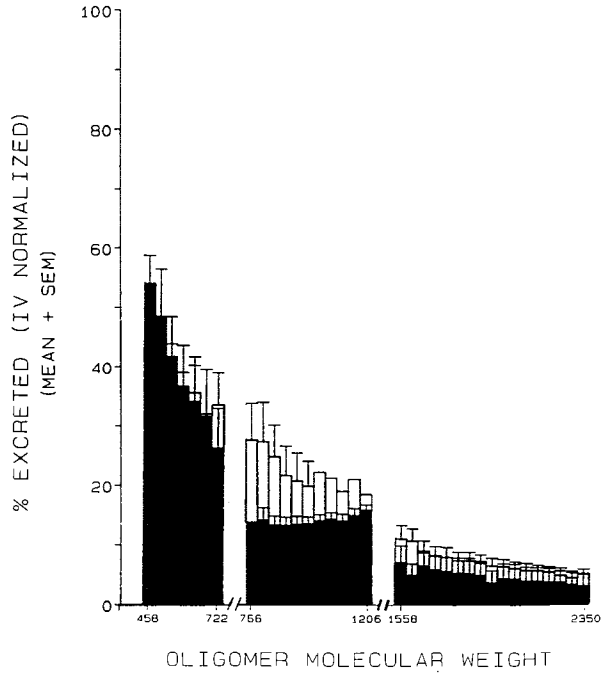

Fig. 1. Excretion of polyethylene glycol + sodium glycocholate following nasal administration. (■) 25% PEG: n = 4 (PEG 600); n = 3 (PEG 1000); n = 3 (PEG 2000). (□) 25% PEG + 1% sodium glycocholate: n = 6 (PEG 600); n = 3 (PEG 1000); n = 4 (PEG 2000). Error bars represent standard error of the mean.

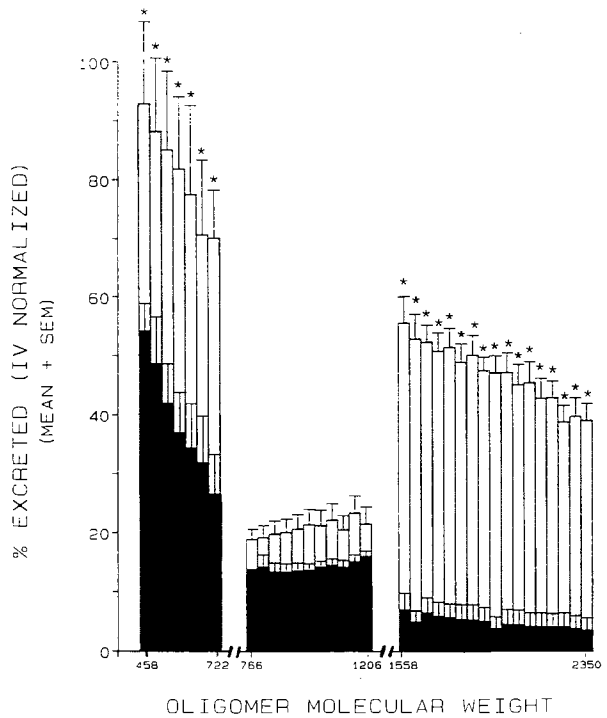

Fig. 2. Excretion of polyethylene glycol + sodium lauryl sulfate following nasal administration. (■) 25% PEG: n = 4 (PEG 600); n = 3 (PEG 1000); n = 3 (PEG 2000). (□) 25% PEG + 1% sodium lauryl sulfate: n = 7 (PEG 600); n = 12 (PEG 1000); n = 3 (PEG 2000). Error bars represent standard error of the mean. (*) A statistically significant difference (P = 0.05) in excretion of PEG and PEG/adjuvant solutions.

posed to a control treatment of a 50-μl instillation of 0.9% sodium chloride to each nostril instead of a PEG/adjuvant solution. The control sample is characterized by closely packed columnar epithelial cells attached to a basement membrane which overlies a loose, highly vascularized connective tissue layer. On the mucosal surface, a lighter staining region composed of a meshwork of cilia, microvilli, and entangled mucus can be seen. Figures 6 and 7 are tissue samples following administration of 25% PEG 600 and 2000 solutions, respectively. Obvious morphologic changes can be seen following exposure to PEG 600. Primarily, one sees disruption of the tight cell–cell packing present in the normal epithelia. The individual cells appear to remain intact, however, as can be seen by the remaining columnar character of the cells and the presence of a ciliated mucosal surface. While these changes indicate that some mucosal alteration is caused by PEG 600 alone, the areas of observed loosened epithelium are interspersed among areas of intact epithelium. While much of the cellular barrier to absorption appears to remain intact, it is difficult to assess how much influence the changes have on the absorption of PEG 600. The tissues exposed to PEG 2000, in comparison, cannot be distinguished from the histological control sample.

The effects of the PEGs in combination with 1% sodium glycocholate on the nasal mucosa are shown in Figs. 8a and b. Again, there is a slight disruption in cell–cell adhesion in the presence of PEG 600. The tissues exposed to PEG 2000 are not visibly altered.

Sodium lauryl sulfate (1%) causes significant alteration of the nasal epithelium as seen in Figs. 9a and b. Changes in the mucosa exposed to PEG 600/1% sodium lauryl sulfate include cellular detachment from the basement membrane, deciliation, and disruption of the columnar cellular structure. Even more striking is the effect of 1% sodium lauryl sulfate on PEG 2000 absorption. In this case, there is virtually no intact epithelium remaining on the surface of the turbinate. The *in situ* studies show greatly enhanced absorption of PEG 600 and 2000 in the presence of 1% sodium lauryl sulfate, which is consistent with the observed loss of the cellular barrier to absorption.

An interesting histological comparison occurs between the effect of 0.1% and that of 1% polyoxyethylene 9 lauryl ether (Figs. 10a–11b). At low concentration, the surfactant actually appears to offset the amount of mucosal alteration generally caused by PEG 600. With PEG 2000 there is a loss of the characteristic columnar structure of the epithelium, but not a loss of cellular integrity. At a 1% concentration, however, dramatic morphologic changes are observed for both molecular weight samples. Some cellular changes, most notably deciliation and disruption at the mucosal surface, are seen following exposure to the PEG 600/1% polyoxyethylene 9 laurel ether mixture. Moreover, a tissue exudate, which may be respiratory mucus, is seen in the airway space (Fig. 11a). One percent polyoxyethylene 9 lauryl ether along with PEG 2000 induces morphologic changes of the same magnitude as those seen with sodium lauryl sulfate. The loss of the surface epithelium again correlates with the *in situ* increase in absorption observed with the PEG 2000 oligomers.

DISCUSSION

The absorption adjuvants chosen for this study may

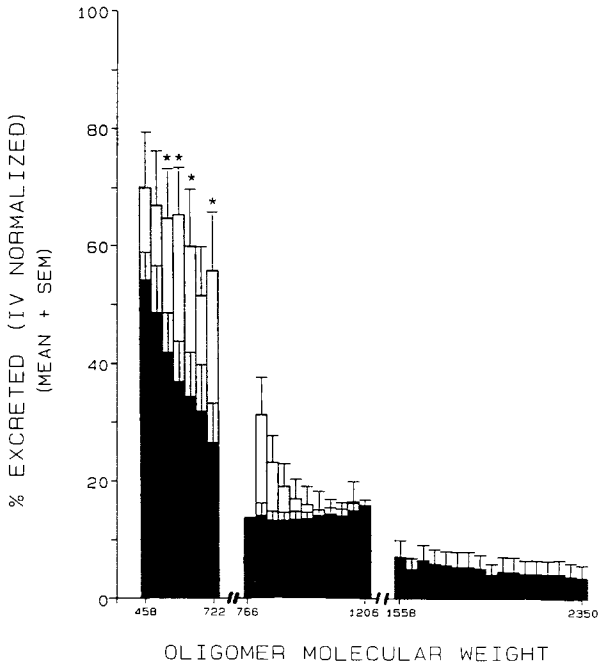

Fig. 3. Excretion of polyethylene glycol + polyoxyethylene 9 lauryl ether following nasal administration. (■) 25% PEG: n = 4 (PEG 600); n = 3 (PEG 1000); n = 3 (PEG 2000). (□) 25% PEG + 0.1% polyoxyethylene 9 lauryl ether: n = 4 (PEG 600); n = 6 (PEG 1000); n = 5 (PEG 2000). Error bars represent the standard error of the mean. (*) A statistically significant difference (P = 0.05) in excretion between PEG and PEG/adjuvant solutions.

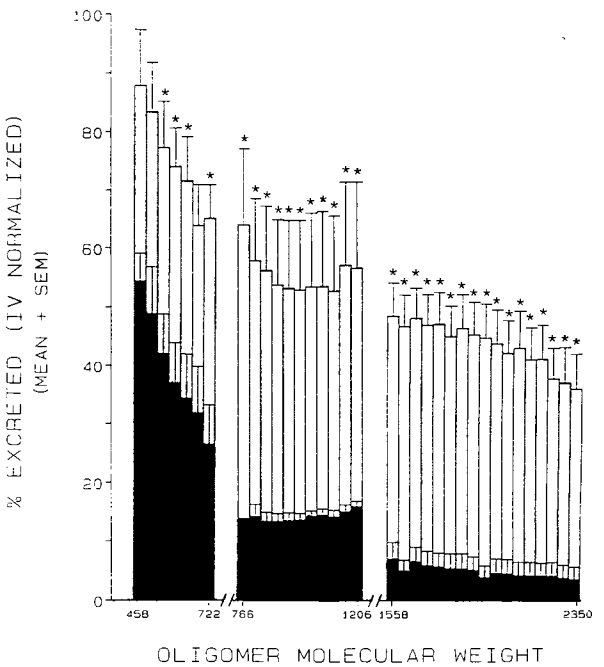

Fig. 4. Excretion of polyethylene glycol + polyoxyethylene 9 lauryl ether following nasal administration. (■) 25% PEG: n = 4 (PEG 600); n = 3 (PEG 1000); n = 3 (PEG 2000). (□) 25% PEG + 1% polyoxyethylene 9 lauryl ether: n = 5 (PEG 600); n = 4 (PEG 1000); n = 5 (PEG 2000). Error bars represent the standard error of the mean. (*) A statistically significant difference (P = 0.05) in excretion between PEG and PEG/adjuvant solutions.

each be acting by different, or combinations of different, mechanisms. Some of these possible mechanisms include (i) influence on the thermodynamic activity of the drug in solution, (ii) alteration of the molecular structure of the cell membrane ranging from temporary membrane pore formation to complete membrane destruction, (iii) loosening of the tight junctions between the epithelial cells, (iv) inhibition of the protease activity present in the mucosa, and (v) alteration of the characteristics of the respiratory mucus in ways which reduce its diffusion barrier properties.

The use of a range of polyethylene glycols to investigate the effects of the absorption adjuvants sodium glycocholate, sodium lauryl sulfate, and polyoxyethylene 9 lauryl ether on the molecular weight permeability profile in the nasal mucosa, when coupled with histologic examinations following adjuvant exposure, can be used to identify some of the mechanisms which may be acting in the case of each enhancer. Sodium glycocholate (1%), for example, has been shown to increase the absorption of insulin (9,10,16–19) and glucagon (20,21) from the nasal cavity. Yet in the PEG studies, there was no change in the molecular weight permeability profile upon addition of the bile salt, and only slight histological changes could be attributed to the sodium glycocholate. This minimal degree of mucosal damage was also observed by Hirata *et al.* (17), who noted slight damage to the surface microvilli following a 1-week treatment with an insulin/1% sodium glycocholate solution. Hirai *et al.* (22) also demonstrated that sodium glycocholate induces only slight cellular disruption in both *in vitro* and *in vivo* tests, yet it does cause a significant inhibitory effect on the activity of leucine aminopeptidase in the nasal mucosa. The inhibitory effect of sodium glycocholate on enzymatic activity has also been demonstrated by Lee and Kashi (23). Such observations suggest that the inhibition of degradative enzymes plays an important role in the adjuvant action of sodium glycocholate, one more influential than any direct action on cell membranes. Since the PEGs are not subject to enzymatic degradation, no increase in their absorption would be expected in the presence of sodium glycocholate via this mechanism. Thus, both the PEG absorption data and the histological evidence suggest that, while sodium glycocholate may be a very biocompatible adjuvant, at this concentration it is not a very effective enhancer for molecules which may

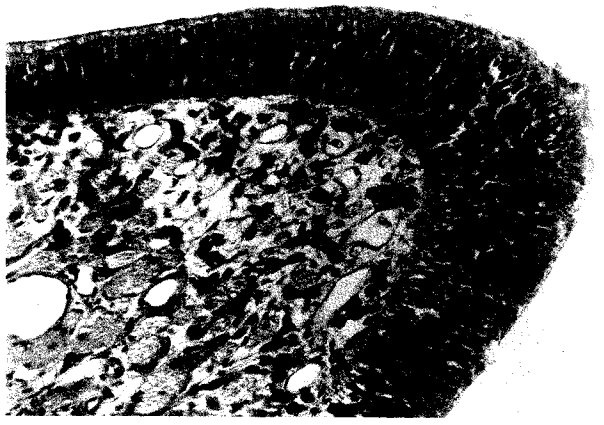

Fig. 5. Nasal mucosa of control (normal saline) animal.

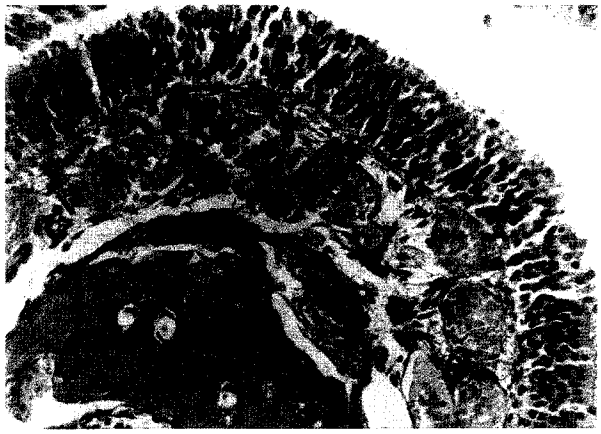

Fig. 6. Nasal mucosa of rat following a 6-hr exposure to 25% PEG 600.

require some alteration in mucosal structure for efficient absorption. Sodium glycocholate may not be entirely biocompatible in whole animals, however, because of its inherent ciliotoxicity (24).

The anionic surfactant, sodium lauryl sulfate, is known to be capable of lysing biological membranes by a mechanism which appears to be a stepwise process involving both lipid solubilization and subsequent protein denaturation and solubilization (25,26). The *in vitro* tests by Hirai *et al.* establish that sodium lauryl sulfate can be extremely damaging to membranes (22). The PEG 600 and 2000 absorption experiments, along with the histological evidence of damage to the epithelium, support the conclusion that 1% sodium lauryl sulfate's adjuvant action is the result primarily of its effects on the cell membrane. The results of the 1% sodium lauryl sulfate/PEG 1000 combination are difficult to explain in light of this mechanism, however. Histological examination of the nasal mucosa following exposure to PEG 1000/1% sodium lauryl sulfate shows morphologic changes of similar magnitude to those seen with PEG 600 and PEG 2000, yet the corresponding absorption results do not show nearly the same degree of absorption enhancement. This suggests that there may be a physicochemical interaction between the sodium lauryl sulfate and PEG 1000 alone or in combination

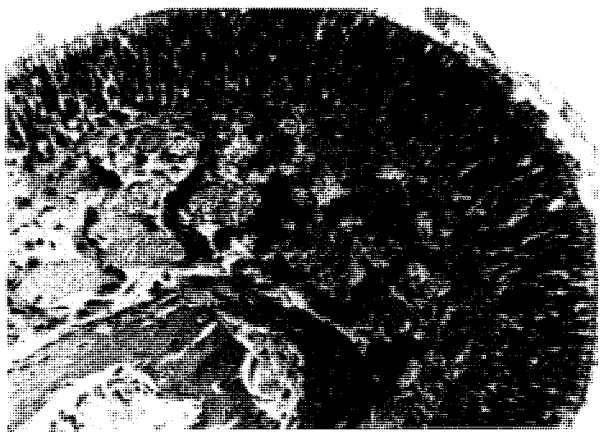

Fig. 7. Nasal mucosa of rat following a 6-hr exposure to 25% PEG 2000.

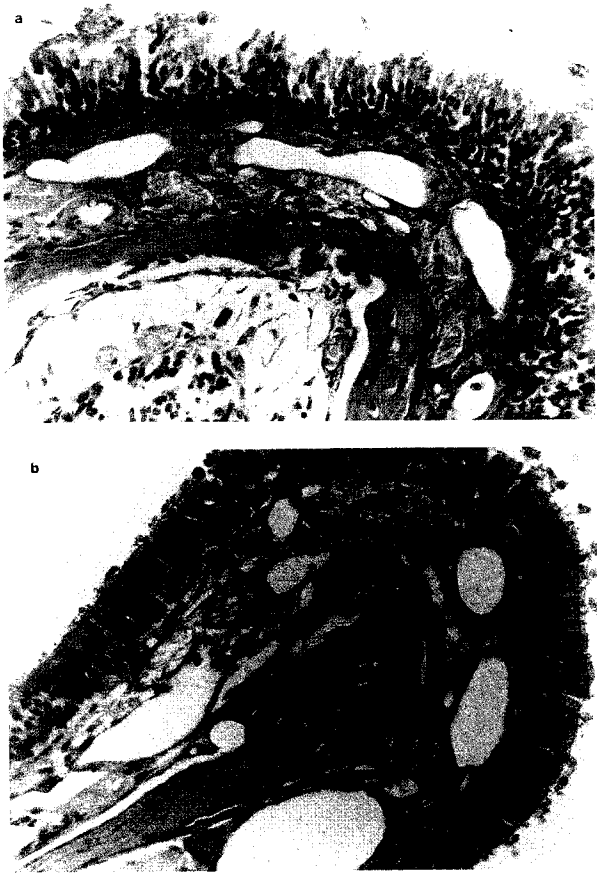

Fig. 8. (a) Nasal mucosa of rat following a 6-hr exposure to 25% PEG 600 + 1% sodium glycocholate. (b) Nasal mucosa of rat following a 6-hr exposure to 25% PEG 2000 + 1% sodium glycocholate.

with constituents of the nasal mucosa which may inhibit the absorption of PEG 1000 regardless of the barrier properties of the epithelia.

Polyoxyethylene 9 lauryl ether (P9E) is also known to solubilize membrane proteins and lipids (27). *In vitro,* it has been associated with increased cellular protein release (less than sodium lauryl sulfate) and the ability to inhibit leucine aminopeptidase (less than sodium lauryl sulfate or sodium glycocholate) (22). It has been shown to cause cellular erosion, cell–cell separation, loss of cilia, and the appearance of a dense mucus layer in live animals (28). These types of histologic changes were also observed in these studies following exposure to 25% PEG 600 or 2000/P9E solutions. The lack of absorption enhancement seen with PEG 2000/0.1% P9E may be associated with the minimal cellular changes seen in the histology samples. The enhanced PEG 600 absorption in the presence of 0.1% P9E, which took place without visually observable alterations in mucosal integrity, is puzzling. Subtle effects on the membrane may be involved, but these or other effects at a molecular level cannot be established from the permeability and histological data alone. These molecular changes may be enough to allow smaller molecules, such as the oligomers of PEG 600, to be increasingly absorbed through the transcellular and/or the paracellular pathways. Yet the results suggest that, what-

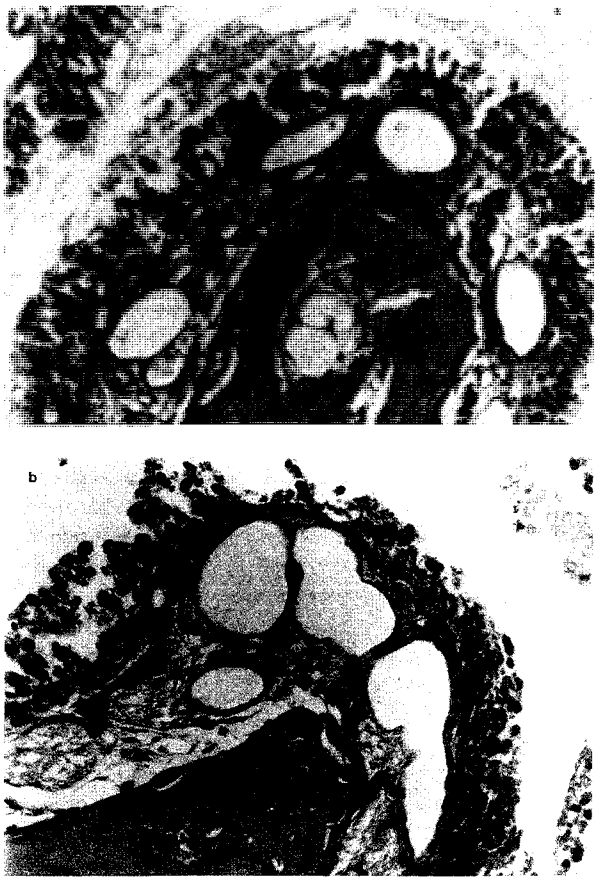

Fig. 9. (a) Nasal mucosa of rat following a 6-hr exposure to 25% PEG 600 + 1% sodium lauryl sulfate. (b) Nasal mucosa of rat following a 6-hr exposure to 25% PEG 2000 + 1% sodium lauryl sulfate.

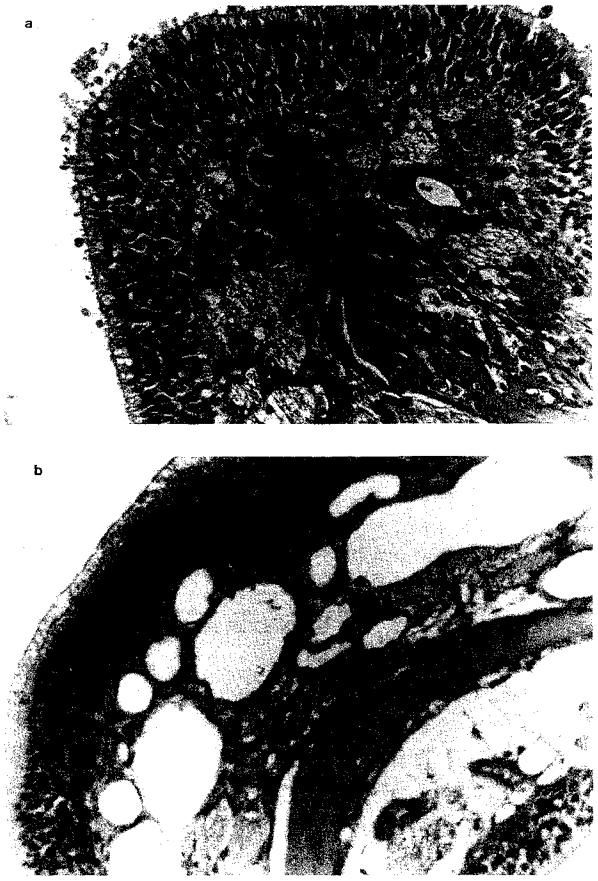

Fig. 10. (a) Nasal mucosa of rat following a 6-hr exposure to 25% PEG 600 + 0.1% polyoxyethylene 9 lauryl ether. (b) Nasal mucosa of rat following a 6-hr exposure to 25% PEG 2000 + 0.1% polyoxyethylene 9 lauryl ether.

ever the changes, they are not of sufficient magnitude to alter the absorption of the larger PEG 1000 and 2000 oligomers. If polyoxyethylene 9 lauryl ether, at a low concentration, is acting on the membrane lipid and protein structure, then this may also account for some of the changes in the columnar structure of the mucosa seen in the PEG 2000 sample. The adjuvant may be altering or extracting the proteins and lipids, thus affecting the geometry of the entire mucosa. When present in higher concentration, the membrane alterations are hardly subtle and a significant loss of cellular integrity is observed. Therefore, it appears that P9E acts primarily on cell membrane structure, and its ability to improve absorption can be correlated with its ability to affect the cellular integrity of the nasal mucosa.

Despite these indications of morphologic changes caused by P9E, it has been used clinically as an adjuvant in intranasal insulin therapy at 0.1–1% concentrations with few apparent adverse effects (29). The differences between the previous observations of morphologic changes caused by P9E and the results of the clinical studies might be explained by differences in the experimental methodologies employed in each of these studies. In the PEG experiments, an isolated nasal cavity system which retains the adjuvant formulation at the absorption site during the entire experiment was used. In the insulin experiments, the subjects were administered aerosol solutions which were allowed to clear from the nasal cavity at normal physiological rates. Such clearance limits the time of exposure to the adjuvant and can be presumed to limit its propensity to damage the mucosa. While the insulin methodology is more clinically relevant, the PEG technique may still be predictive of morphologic changes which could occur with chronic, multidose therapy.

SUMMARY

Changes in the size discrimination capabilities of the cell membranes or paracellular channels induced by an absorption adjuvant should result in a corresponding change in the molecular weight–permeability profile. These changes could range from increasing the absorption of larger molecules relative to smaller ones or to complete elimination of the molecular size dependency of absorption. The results from the PEG absorption and histology studies indicate that appreciable absorption of these large, hydrophilic molecules through the nasal mucosa occurs when cell–cell adhesion is reduced, thus increasing transport through the paracellular pathways to the underlying vasculature, or when the surface epithelium is removed thus eliminating much of the barrier to systemic absorption.

It was found that each of the adjuvants used in this study has a unique effect on the molecular weight perme-

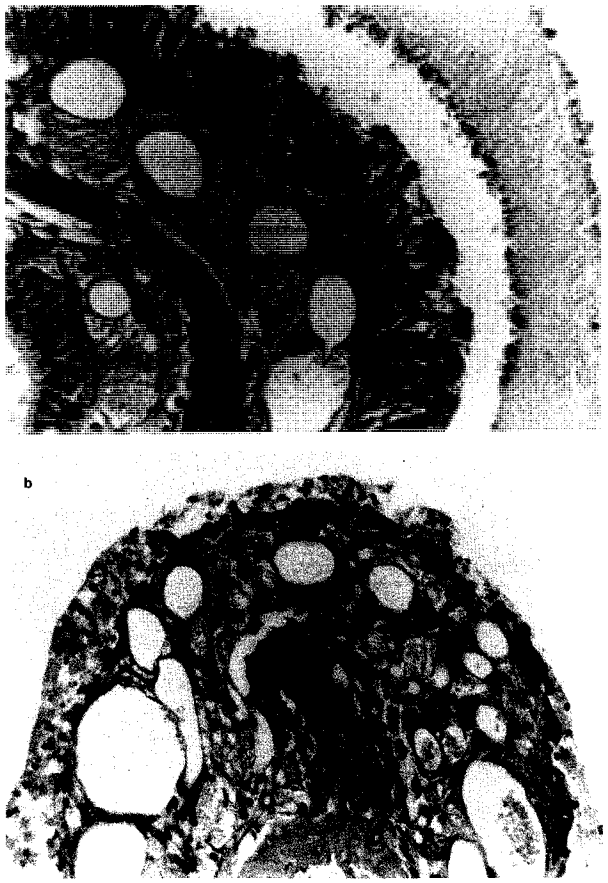

Fig. 11. (a) Nasal mucosa of rat following a 6-hr exposure to 25% PEG 600 + 1% polyoxyethylene 9 lauryl ether. (b) Nasal mucosa of rat following a 6-hr exposure to 25% PEG 2000 + 1% polyoxyethylene 9 lauryl ether.

ability profile of the nasal mucosa and on the integrity of the mucosa itself. The bile salt, sodium glycocholate, does not significantly alter the permeability or the structure of the mucosa. The anionic surfactant, sodium lauryl sulfate, and the nonionic surfactant, polyoxyethylene 9 lauryl ether, markedly increase permeability and profoundly alter the mucosa. Results showing that PEG 900 is absorbed primarily via paracellular routes in the small intestine have been reported by Hollander *et al.* (30). The relationships between the degree of absorption enhancement of the PEGs and the histological state of the nasal mucosa observed in these studies suggest that the molecular weight permeability profile of polyethylene glycol, a passively absorbed, hydrophilic molecule, may be used to infer mechanistic processes related to the absorption enhancement which occurs with adjuvant-acting agents.

Table I. Summary of Mucosal Integrity Following Exposure to PEG/Adjuvant Mixtures[a]

Mixture	PEG 600 (25%)	PEG 2000 (25%)
No enhancer	++	0
1% sodium glycocholate	++	0
1% sodium lauryl sulfate	+++	+++
0.1% P9E	0	+
1% P9E	++	+++

[a] 0.9% NaCl control value = 0. Grading criteria: (0) no apparent cellular changes; (+) some cellular disruption, no significant loss of mucosal integrity; (++) moderate mucosal damage, some loss of surface epithelium; (+++) severe damage, substantial loss of surface epithelial layer.

ACKNOWLEDGMENTS

This paper was presented at the American Association of Pharmaceutical Scientists Third National Meeting, Orlando, FL, October 1988. Maureen Donovan gratefully acknowledges the support of an American Foundation for Pharmaceutical Education Fellowship and a Rackham Predoctoral Fellowship. Additional support for this research was provided by the Rugby-Darby Group Companies. The authors also wish to thank Mr. Gary DeBrincat for his technical assistance and Ms. R. Kaye Brabec of the University of Michigan Morphology Core Unit for her expertise in the preparation of the histology samples.

REFERENCES

1. J. Tonndorf, R. W. Hyde, H. I. Chinn, and J. E. Lett. *Ann. Otol. Rhinol. Laryngol.* 62:630–639 (1953).
2. Y. Kaneo. *Acta Pharm. Suec.* 20:379–388 (1983).
3. A. A. Hussain, S. Hirai, and R. Bawarshi. *J. Pharm. Sci.* 68:1196 (1979).
4. R. T. Woodyatt. *J. Metab. Res.* 2:793 (1922).
5. M. D. Donovan, G. L. Amidon, and G. L. Flynn. *Pharm. Res.* 7:863–868 (1990).
6. C. McMartin, L. E. F. Hutchinson, R. Hyde, and G. E. Peters. *J. Pharm. Sci.* 76:535–540 (1987).
7. A. N. Fisher, K. Brown, S. S. Davis, G. D. Parr, and D. A. Smith. *J. Pharm. Pharmacol.* 39:357–362 (1987).
8. Y. Maitani, Y. Machida, and T. Nagai. *Int. J. Pharm.* 49:23–27 (1989).
9. S. Hirai, T. Yashiki, and H. Mima. *Int. J. Pharm.* 9:165–172 (1981).
10. 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).
11. J. P. Longenecker, A. C. Moses, J. S. Flier, R. D. Silver, M. C. Carey, and E. J. Dubovi. *J. Pharm. Sci.* 76:351–355 (1987).
12. S. C. Raehs, J. Sandow, K. Wirth, and H. P. Merkle. *Pharm. Res.* 5:689–693 (1988).
13. M. Mishima, Y. Wakita, and M. Nakano. *J. Pharmacobiodyn.* 10:624–631 (1987).
14. P. Tengamnuay and A. K. Mitra. Presented at the American Association of Pharmaceutical Scientists 3rd Annual Meeting, Orlando, FL, 1988.
15. A. Hussain, S. Hirai, and R. Bawarshi. *J. Pharm. Sci.* 69:1411–1413 (1980).
16. S. Hirai, T. Ikenaga, and T. Matsuzawa. *Diabetes* 27:296–299 (1978).
17. Y. Hirata, T. Yokosuka, T. Kasahara, M. Kikuchi, and K. Ooi. *Excerpt. Med. Int. CS* 468:319–326 (1979).
18. B. J. Aungst, N. J. Rogers, and E. Shefter. *J. Pharmacol. Exp. Ther.* 244:23–27 (1988).
19. A. E. Pontiroli, M. Alberetto, A. Secchi, G. Dossi, I. Bosi, and G. Pozza. *Br. Med. J.* 284:303–306 (1982).
20. A. E. Pontiroli, M. Alberetto, and G. Pozza. *Br. Med. J.* 287:462–463 (1983).
21. A. E. Pontiroli, M. Alberetto, and G. Pozza. *Acta Diabetol. Lat.* 22:103–110 (1985).
22. S. Hirai, T. Yashiki, and H. Mima. *Int. J. Pharm.* 9:173–184 (1981).

23. V. H. L. Lee and S. D. Kashi. *Proc. Int. Symp. Control. Rel. Bioact. Mater.* 14:53–54 (1987).
24. G. S. M. J. E. Duchateau, J. Zuidema, and F. W. H. M. Merkus. *Int. J. Pharm.* 31:193–199 (1986).
25. F. H. Kirkpatrick and H. E. Sandberg. *Biochim. Biophys. Acta* 298:209–218 (1973).
26. F. H. Kirkpatrick, S. E. Gordesky, and G. V. Marinetti. *Biochim. Biophys. Acta* 345:154–161 (1974).
27. B. J. Aungst and N. J. Rogers. *Pharm. Res.* 5:305–308 (1988).
28. W. A. Lee, R. E. Ennis, and L. C. Foster. *Proc. Int. Symp. Control. Rel. Bioact. Mater.* 15:70–71 (1988).
29. R. Salzman, J. E. Manson, G. T. Griffing, R. Kimmerle, N. Ruderman, A. McCall, E. I. Stoltz, C. Mullin, D. Small, J. Armstrong, and J. C. Melby. *N. Engl. J. Med.* 312:1078–1084 (1985).
30. D. Hollander, S. Koyama, V. Dadufalza, D. Q. Tran, P. Krugliak, T. Ma, and K.-Y. Ling. *J. Lab. Clin. Med.* 113:505–515 (1989).