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Report

Absorption of Polyethylene Glycols 600 Through 2000: The Molecular Weight Dependence of Gastrointestinal and Nasal Absorption

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Polyethylene glycols (PEGs) 600, 1000, and 2000 were used to study the molecular weight permeability dependence in the rat nasal and gastrointestinal mucosa. Absorption of the PEGs was measured by following their urinary excretion over a 6-hr collection period. HPLC methods were used to separate and quantitate the individual oligomeric species present in the PEG samples. The permeabilities of both the gastrointestinal and the nasal mucosae exhibited similar molecular weight dependencies. The steepest absorption dependence for both mucosae occurs with the oligomers of PEG 600, where the extent of absorption decreases from approximately 60% to near 30% over a molecular weight range of less than 300 daltons. Differences in the absorption characteristics between the two sites appear in the molecular weight range spanned by PEG 1000. For these oligomers, the mean absorption from the nasal cavity is approximately 14%, while that from the gastrointestinal tract is only 9%. For PEG 2000, mean absorption decreases to 4% following intranasal application and below 2% following gastrointestinal administration. Within the PEG 1000 and 2000 samples, however, very little molecular weight dependency is seen among the oligomers. In the range studied, a distinct molecular weight cutoff was not apparent at either site.

KEY WORDS: polyethylene glycol; nasal absorption; gastrointestinal absorption; molecular weight-dependent absorption; molecular weight cutoff; permeability.

INTRODUCTION

The dependence of transmembrane permeability on molecular size has been studied using compounds of vastly different physicochemical properties (1). While a compound's molecular weight is often predictive of its degree of absorption across many biological membranes (2), additional molecular parameters such the partition coefficient or solubility can significantly improve the structure–permeability correlations (1,3). If the properties of the molecular weight dependency of permeation and subsequent absorption are to be more clearly established, it is important to use model compounds that are physicochemically similar to one another in order to limit the need to include these additional parameters. Polydisperse fractions of high molecular weight dextrans and dextran derivatives (3000–70,000 daltons) have been used previously as physicochemically similar gastrointestinal and nasal permeability markers (4,5).

Chadwick *et al.* (6) also identified the polyethylene glycols (PEGs), polydisperse polymeric mixtures available in a wide range of molecular weights, as a series of compounds useful in the investigation of molecular weight dependencies. As a polyoxyethylene polymer, the physicochemical properties, particularly the partition coefficient of the PEGs, do not change as drastically with increasing molecular size as happens within a series of alkyl homologues (7). Chadwick *et al.* (6) used polyethylene glycol 400 as an absorbable marker compound, yet polyethylene glycol 4000 is commonly used as a nonabsorbable gastrointestinal marker. These observations strongly suggest a significant molecular weight effect, rather than physicochemical component, controlling the permeability of the polyethylene glycols (PEGs) in the gastrointestinal tract. The PEGs also appear to be good markers because of their high solubilities (8) and low toxicities (9,10).

The PEGs are rapidly excreted and not subject to tissue or bacterial metabolism. This rapid excretion allows their total absorption to be measured by following their appearance in the urine. After intravenous administration, 77% of PEG 400, 85% of PEG 1000, and 96% of PEG 6000 were recovered from the urine within 12 hr (11,12). In an oral dosing study of PEG 400 in human volunteers, 92.8% of a 10-g dose was recovered unchanged over a 4-day collection period. Of this, 58.5% was recovered from the urine and 34.3% from the feces (6).

Using the PEGs as marker compounds, a direct comparison between the molecular weight sensitivities of permeability in the nasal and gastrointestinal epithelia can be

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made. Differences between these two absorption sites favoring the absorption of larger molecules from the nasal mucosa may support the current interest in the delivery of systemic medications, macromolecules in particular, via the nasal route.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing from 250 to 350 g (9 to 13 weeks old) were used in a series of gastric, intravenous, and nasal absorption studies. All animal procedures were approved by the University of Michigan Committee for the 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, St. Louis, MO). To maintain normal body temperatures, all animals were placed on warming blankets for the duration of each experiment. A hypertonic aqueous solution (polymer dissolved in 0.9% NaCl) of either PEG 600, PEG 1000, or PEG 2000 (Sigma Chemical, St. Louis, MO, and BASF, Parsippany, NJ) was administered such that each rat received a 25-mg dose of the given polymer sample. The absorption of each series of oligomers was determined by measuring the urinary excretion of polyethylene glycol over the 6-hr interval following dosing. Adequate urine output was ensured by the intravenous administration of 0.5 ml of heparinized normal saline (10 U/ml) every 30 min throughout the experiment through a femoral artery cannula of PE-50 tubing (Intramedic, Clay Adams, Parsippany, NJ). In order to collect the entire volume of urine excreted, the bladder of each rat was cannulated with a section of polyethylene tubing (PE-100, Intramedic, Clay Adams, Parsippany, NJ) using a purse string suture. The urine was collected into borosilicate glass test tubes and stored at −20°C prior to analysis.

Nasal Absorption Studies

The nasal cavity of each rat was isolated from the remainder of its respiratory tract and gastrointestinal tract in order to eliminate mechanical losses of the instilled dose during the experiment. The procedure used was a modification of the technique originated by Hirai *et al.* (13). Isolation was accomplished by making a midline incision in the neck to expose the trachea and esophagus. A small incision in the trachea was made, and a piece of PE-100 tubing was inserted from the trachea toward the lungs to provide a patent airway. Following this placement, the trachea was severed above the tubing incision. The esophagus was also tied off with suture and severed. The entire pharyngeal area was then closed off by tying another suture above the level of the larynx. The nasopalatine tract was sealed off with a drop of cyanoacrylate glue. A 50-μl volume of PEG solution (25% w/v) was placed into each nostril using a piece of PE-10 tubing attached to a 0.5-ml syringe (Becton–Dickinson, Rutherford, NJ). The tubing was gently inserted into the nostrils a distance of 0.5–1.0 cm, followed by the delivery of the solution to the nasal cavity. A similar modification of the Hirai technique has been reported (14), and no differences in nasal absorption or loss to the gastrointestinal tract were observed with this method when compared to the original technique.

Gastrointestinal Absorption Studies

For the oral dosing studies, the rats were fasted for 12 to 18 hr prior to the experiment. Water was provided *ad libitum*. The gastrointestinal dose of PEG was administered as a 5% (w/v) solution via a gavage needle into the stomach. Administration of the marker compound was followed by a small volume of normal saline in order to flush the entire dose from the syringe and gavage needle.

Intravenous Studies

Five percent (w/v) solutions of PEG prepared in heparinized normal saline were infused into the femoral artery cannula at a rate of approximately 0.5 ml/min. After the infusion, the tubing was flushed with heparinized saline.

Assay and Extraction Procedures

High-performance liquid chromatography (HPLC) has been used to separate and quantitate the individual oligomers of a broad spectrum of polyethylene glycols (15). Since PEGs contain no chromophoric groups and derivitization procedures are often cumbersome and incomplete, monitoring changes in effluent refractive index was chosen as the method of detection.

A chromatographic system consisting of a Model 6000A solvent delivery system (Waters and Associates, Milford, MA), WISP 712 (Waters and Associates, Milford, MA) or Rheodyne 7125 (Rheodyne Inc, Cotati, CA) injection system, and a Model 410 differential refractometer and column heater module (Waters and Associates, Milford, MA) was used for all polyethylene glycol assays. The refractometer and column heater module were programmed to maintain a constant temperature of 40°C.

Polyethylene glycol 600 (Sigma Chemical, St. Louis MO) and PEG 1000 and 2000 (Pluracol E1000 and E2000, respectively; gifts from BASF, Parsippany, NJ) were separated from urine using a multiple organic-phase extraction procedure. Each urine sample was diluted to 4 ml with distilled water. Ten milliliters of anhydrous ethyl ether (Baker Chemical, Phillipsburg, NJ) was added to each urine sample. The entire volume was vortexed for 30 sec. The ether phase was removed and replaced with a 10-ml aliquot of hexane (Fisher Scientific, Fair Lawn, NJ). The sample was shaken briefly followed by removal of the hexane phase. Approximately 4 ml of Amberlite MB-3 mixed bed ion-exchange resin (Sigma Chemical, St. Louis, MO) was added to the remaining aqueous urine samples. Each sample was mixed on a rocking-type shaker (Labquake, Labindustries, Berkeley, CA) for at least 10 min. The entire sample, along with the resin, was placed on a prewashed (with methylene chloride) 5-ml Extrelut QE extraction cartridge (EM Science, Gibbstown, NJ). The polyethylene glycols were eluted from the cartridge using six 8-ml aliquots of methylene chloride (Fisher Scientific, Fair Lawn, NJ). The methylene chloride samples were evaporated in a HaakeBuchler Vortex-Evaporator (Model 4322, Saddle Brook, NJ). Each vial was rinsed with 5 ml of methanol, followed by the evaporation of this phase. The samples were reconstituted in the appropriate mobile phase, filtered through a Millipore HV_4 4-mm filter (Millipore Corp., Milford, MA), and assayed as de-

scribed in Figs. 1a–c. Assay standards were prepared by spiking known quantities of PEG 600, 1000, or 2000 into blank rat urine and then extracting the PEGs as described. In order to quantitate the relative amount of each oligomer present, an external standard was added to each sample prior to assay. Propyl *p*-aminobenzoate (Sigma Chemical, St. Louis, MO), pentyl *p*-aminobenzoate, and hexyl *p*-aminobenzoate (synthesized) were used as the standards for PEG 600, 1000, and 2000, respectively.

Data Analysis

Ratios of each oligomer peak height to the peak height of a constant amount of external standard were plotted against the known total polymer concentration. Linear regression equations were calculated for each oligomer. The peak ratios for each oligomer in the unknown sample were calculated and converted to the relative amount of PEG present using the regression equations.

RESULTS

As previously noted by Tagesson and Sjodahl (15), the excretion of the PEGs shows a molecular weight dependency. This is thought to be due to increased systemic capillary filtration of the lower molecular weight oligomers, which results in a larger volume of distribution and, correspondingly, a slower clearance. Therefore, the excretion of the PEGs was studied following intravenous administration, in addition to nasal and gastrointestinal administration, to account for these changes in clearance with molecular weight. The excretion of the PEG oligomers following nasal or GI administration was normalized to this intrinsic molecular weight dependency as follows:

$$\%\ \text{dose excreted}_{(\text{normalized})} = \frac{\%\ \text{dose excreted}_{(\text{nasal or GI})}}{\%\ \text{dose excreted}_{\text{iv}}} \times 100\% \quad (1)$$

Figures 2 and 3 show the results of the molecular weight cutoff experiments. Overall, the percentage of dose excreted over a 6-hr period for PEGs 600 through 2000 following intravenous administration increases with increasing molecular weight (Fig. 2). The mean values were the values used to normalize the nasal and gastrointestinal results. As can be seen from Fig. 3, the molecular weight dependency of absorption in the lower molecular weight region (PEG 600) following gastrointestinal administration is quite steep. The mean excretion of these oligomers over 6 hr decreases from 60 to 35% of the amount administered within a molecular weight range of 300 daltons. For PEG 1000, the percentage excreted is further reduced, and in contrast to the PEG 600 results, all of the oligomers are excreted to nearly the same extent (9%). The absorption of the oligomers of PEG 2000 is also virtually independent of size. However, the overall value of the excretion of these oligomers is decreased to approximately 1.8%.

The corresponding results for the absorption of PEG 600, 1000, and 2000 following nasal administration are also shown in Fig. 3. The overall shape of the molecular weight absorption profile is similar to that of the gastrointestinal tract. Again, the steepest molecular weight dependency is seen with the sample of oligomers designated as PEG 600. Statistically significant differences in the absorption values between the nasal and the gastrointestinal mucosa become apparent when the higher molecular weight samples are studied. The mean absorption for the oligomers of PEG 1000 is approximately 50% higher for the nasal mucosa than for the gastrointestinal tract (14 vs 9%), and the mean absorption for the oligomers of PEG 2000 is over twice that of the gastrointestinal mucosa.

DISCUSSION

The molecular weight dependence of gastrointestinal permeability has been determined for lower molecular weight PEGs in humans and various animal species (6,15–21), but a distinct molecular weight cutoff has not been defined. The common use of ^{14}C-PEG 4000 as a nonabsorbable gastrointestinal marker along with the use of PEG 400, 600, and 1000 to study permeability changes in the GI tract suggests that the cutoff for PEG should occur between 1500 and 4000 daltons. In this study, a broader and higher molecular weight spectrum of PEGs was used in a single species (rat), to identify the molecular weight cutoff for absorption. The results obtained agree with data reported in several other species which show a steep absorption dependence in the molecular weight range of 400 to 1000 daltons and an asymptotic oral absorption value of approximately 2% above 1300 daltons (15,18–20).

While the molecular weight dependencies for the GI and nasal absorption profiles appear qualitatively similar, differences in the permeabilities of these mucosae become apparent when the mass transport characteristics of each route are evaluated quantitatively. Assuming that PEG is passively absorbed from both anatomical regions, Fick's first law ([Eq. (2)] can be used to describe its absorption.

$$\frac{dM}{dt} = -P_{\text{mem}} A \Delta C \quad (2)$$

where M signifies the total mass transported through membrane; t, the time; P_{mem}, the membrane permeability; A, the surface area for absorption; and ΔC, the concentration gradient across the membrane.

Since the absorptive surface area of the rat intestine is approximately 700 cm^2 (22) and the surface area of the nasal cavity is approximately 10 cm^2 (23), if the membrane permeabilities were similar, absorption from the gastrointestinal tract would be expected to exceed that from the nasal cavity. Yet the total mass of polyethylene glycol absorbed from the nasal cavity was greater than or equal to the mass absorbed from the gastrointestinal tract for all of the oligomers measured. For the oligomers of PEG 2000, assuming no significant dilution by secretions occurs at either site and that sink conditions occur in the vasculature, the concentration gradient and the surface area can be treated as constant values. These assumptions are valid since the total mass absorbed is less than 4% of the dose administered, and during the 6-hr time interval the polymer solutions are probably well dispersed throughout the entire nasal cavity or the gastrointestinal tract. The results show that the absorption of the oligomers larger than 2000 daltons is approximately 2.3 times

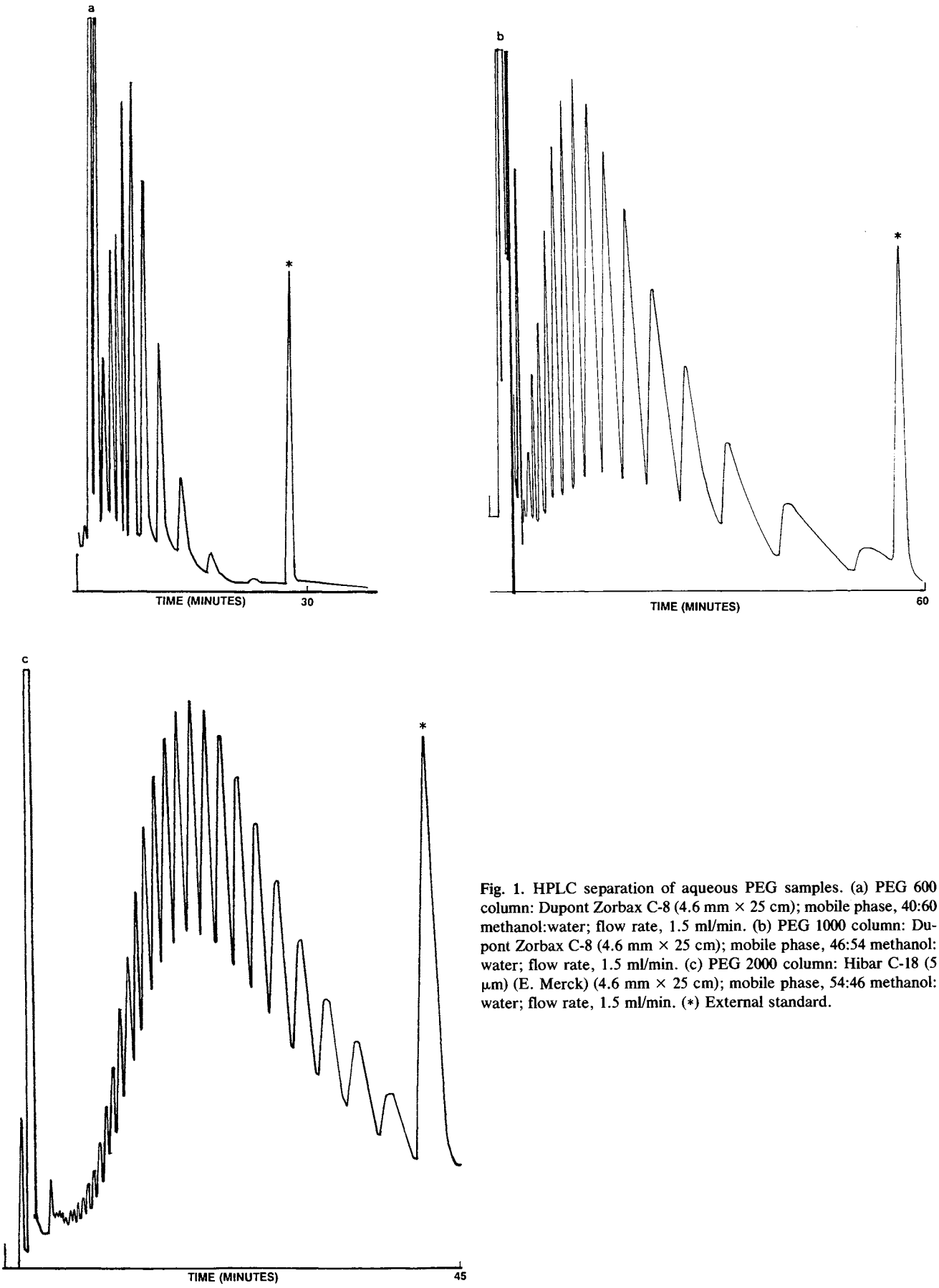

Fig. 1. HPLC separation of aqueous PEG samples. (a) PEG 600 column: Dupont Zorbax C-8 (4.6 mm × 25 cm); mobile phase, 40:60 methanol:water; flow rate, 1.5 ml/min. (b) PEG 1000 column: Dupont Zorbax C-8 (4.6 mm × 25 cm); mobile phase, 46:54 methanol: water; flow rate, 1.5 ml/min. (c) PEG 2000 column: Hibar C-18 (5 μm) (E. Merck) (4.6 mm × 25 cm); mobile phase, 54:46 methanol: water; flow rate, 1.5 ml/min. (*) External standard.

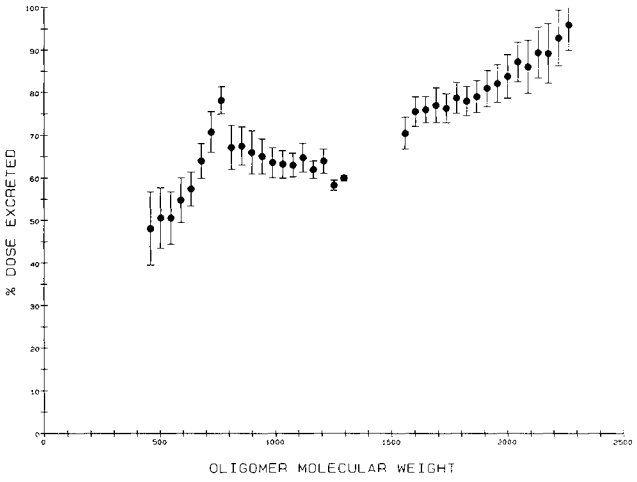

Fig. 2. Excretion of oligomers of PEG 600–2000 following intravenous administration in the rat. Error bars represent the standard error of the mean. n = 6 (PEG 600), 4 (PEG 1000), and 7 (PEG 2000).

greater following nasal than gastrointestinal administration. While the initial concentration administered gastrointestinally was five times less than that used nasally, if the permeabilities of these two mucosae were indeed equal, a 30-fold dilution of the administered dose would have had to occur in the gastrointestinal tract in order to satisfy Eq. (2). This situation is physically unrealistic since, in order to result in a dilution of this magnitude, the internal volume of the rat gastrointestinal tract would have to be nearly 15 ml. The observed total gastrointestinal volume in rats of the size used is approximately 3 ml.

No similar quantitative comparisons can be made for the oligomers of PEG 600 or 1000 since the assumption of a constant concentration gradient does not hold in these cases.

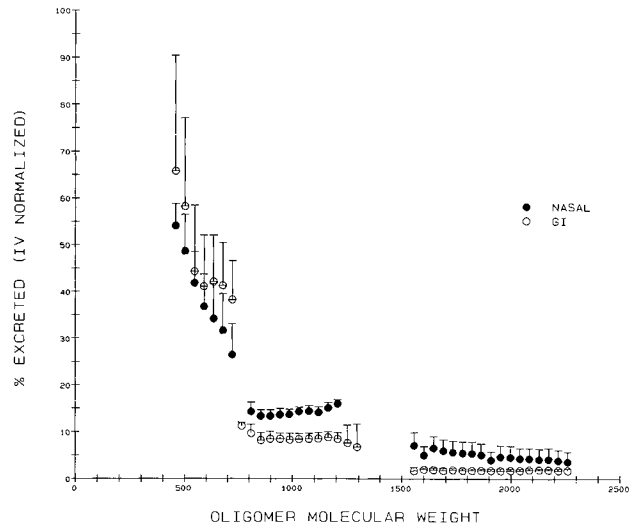

Fig. 3. Excretion of oligomers of PEG 600–2000 following nasal and gastrointestinal administration in the rat (normalized to mean intravenous excretion values). Error bars represent the standard error of the mean. Nasal: n = 4 (PEG 600), 3 (PEG 1000), and 3 (PEG 2000). GI: n = 8 (PEG 600), 4 (PEG 1000); and 3 (PEG 2000).

The nearly equal total absorption of the oligomers from both sites again provides supportive evidence for the existence of significant differences in the permeability characteristics between the two mucosae in light of the magnitude of the difference between the absorptive surface areas. In addition, observations in our own laboratory have shown very little difference in the absorption of the PEGs after oral administration when the animals were kept hydrated by gavaging normal saline into the stomach, further diluting the PEGs, rather than via the arterial catheter. This suggests that the concentration gradient does not play a major role in determining the total absorption of these compounds under these experimental conditions.

Regardless of the quantitative role of the concentration gradient, the magnitude of the gradient present at the mucosal surface should have no effect on the absorption of the oligomers relative to one another. Therefore, the shape of the observed molecular weight–absorption profiles is characteristic of the individual mucosa. Absorption from both the nasal and the gastrointestinal mucosae exhibits a strong dependence on the molecular weight of the PEGs. While a molecular weight cutoff cannot be identified at either site, the intrinsic PEG molecular weight–absorption profiles suggest that obtaining clinically useful bioavailabilities for compounds whose molecular weights exceed 2000 daltons from either the GI tract or the nasal mucosa, without some alteration in the barrier properties of the mucosa, is unlikely.

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