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Measurement of Ileal Permeability with Different-sized Polyethylene glycols (PEG 400, 600, and 1000)

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Polyethylene glycols (PEGs; 400, 600, and 1000) were used to study the molecular weight (MW) permeability dependence in the rat ileal mucosa. Absorption of the PEGs was measured by following their recirculation perfusion over a 3 hr collection period. HPLC methods were used to separate and quantitate the individual oligomers present in the solution of PEGs mixtures (MW range 330 to 1122 D). In the range studied, a distinct molecular weight cutoff was not identified. Corrected for the length of ileum used in the study, over the molecular weight range 330 to 1122 D, the apparent permeability (P_{app}) of PEG ranged from $3.2 \pm 0.06 \times 10^{-5}$ cm/sec (mean $\pm$ SEM, n=7) to $0.1 \pm 0.02 \times 10^{-5}$ cm/sec. Also, it was observed that the apparent permeability was inversely proportional to approximately $MW^{2.4}$.

Key words : Intestinal permeability, Polyethylene glycols, Small intestine

INTRODUCTION

The small intestine, a highly selective organ, absorbs nutrients, water, and electrolytes with a high degree of efficiency and simultaneously delays and inhibits the absorption of large and potentially harmful antigenic or injurious molecules. Intestinal permeability relates to the selective ability of the intestinal epithelium. Recently, there has been an explosion of new information concerning the selective permeability characteristics of the intestine. Clinical studies have shown altered intestinal permeability in a number of disorders, including Crohn's disease (Sanderson *et al.*, 1987), celiac sprue (Jenkins *et al.*, 1986), rheumatoid arthritis (Jenkins *et al.*, 1986), and indomethacin-associated enteritis (Bjarnason *et al.*, 1987).

A variety of compounds have been used to delineate the permeability characteristics of mucosal membranes in health and disease (Tagesson *et al.*, 1978; Bjarnason, 1994; Bode *et al.*, 1991). These include urea, erythritol, mannitol, lactulose, inulin, and creatinine (Fordtran, 1965; Peeters, 1994; Bright-Asare and Bider, 1973). A new approach to the measurement of intestinal permeability using low molecular weight polyethylene glycol(PEG) in man and animals has been developed by many investigators (Chadwick, 1977 a; b; Oliva *et al.*, 1994). Chadwick and his colleagues (1977a; b) introduced a mixture of low molecular weight polyethylene glycols as 'ideal' probe molecules for measuring intestinal permeability: Polyethylene glycol is water soluble, nontoxic, not degraded by intestinal bacteria, not metabolized during or after passage through the intestinal wall, and rapidly excreted in the urine in a measurable form. The different-sized molecular compounds cross the intestinal epithelium at different rates, allowing characterisation of the passive permeability properties of the mucosa. The PEG method offers a simple, harmless and reproducible method to measure intesinal permeability properties.

At this point, there are experimental informations about the PEGs permeation across jejunal epithelium of small intestine. However, there is little information about how different-sized PEGs (400, 600, and 1000) are absorbed by the ileum. Because the lower part of small intestine is also commonly involved in inflammatory bowel disease, the basic characteristics of PEGs (MW range 330 to 1122 D) permeation *in situ* in perfused ileal segments of rats were studied. It has been also proposed that the aqueous pores/channels of the jejunum have an effective diameter of 670-880 μm and that of the ileum of 290-380 μm (Fordtran, 1965). This would suggest that the aqueous pore/channel size distribution changes from jejunum to ileum, in which case one would expect that the ratio of jejunal/ileal permeability would increase on increasing molecular weight.

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Therefore, in this study I was interested whether the aqueous pore/channel size distribution varies depending on the different site of intestine. The mixture of polyethylene glycol (400, 600, and 1000) having different molecular weight/size was chosen to investigate permeability characteristics as a function of molecular weight or size in the lower part of small intestine (ileum).

MATERIALS AND METHODS

Surgical procedures

After an overnight fast, male rats (CD-Crl : CD(SD) BR, Charles River Breeding Laboratories, Ltd. U.K.) weighing 250-300 g were anaesthetized (18 mg/kg B. W.) by i.v. pentobarbital (Sagatal; May & Baker, U.K.) injection and body temperature was maintained at 37°C using an electric heating pad. Following leparotomy, a 20 cm length of terminal ileum proximal to the caecum with intact mesenteric vasculature were cannulated at both ends and returned to the peritoneal cavity without disrupting the blood flow. The length of ileal segment used was measured using a standard silk thread. Anaethesia was maintained by administration of i.v. pentobarbital (6 mg/kg B.W.) every 15 min throughout the experimental period.

Experimental protocol

Before the experiment, 20 mL of the appropriate perfusing solution was placed in reservoir (pH 7.4). After 15 min equilibration period, a 200 μL perfusate sample was withdrawn from the reservoir at the first 10 min and then every 30 min for 3 hr. At the end of the experiment the animals were killed by anaesthetic overdose and the perfused intestinal loops were removed. The results were standardized per cm of tissue length.

Perfusion details

The following was applied to ileum in each animal using an in situ perfusion technique : The cannulae from the intestinal loop were connected by teflon tubing to the perfusing solution reservoir maintained at 37°C. A peristaltic perfusion pump (Anachem, U.K.) recirculated solution through intestinal loop at a flow rate of 0.5 ml/min (a rate chosen based on preliminary experiments). The perfusing solution was gassed with O_2 95% : CO_2 5%(V/V) additionally containing 5 g/l of polyethylene glycol (PEG) 4000 with 5 μCi ^{14}C-PEG 4000 (Amersham, U.K.) as a non-absorbable marker for fluid transport. A mixture of 5% polyethylene glycols (PEG 400/600/1000; 1/2/20 ratio) in isotonic electrolyte solution (Table I) was perfused through the ileum at 37°C. The ratio of the batches of PEG was chosen to provide similar peak heights of the oligomers after HPLC.

Table I. Composition of isotonic electrolyte perfusion solution (m mol/l)

Sodium Chloride	25	Sodium Sulphate	40
Potassium Chloride	10	Sodium Bicarbonate	20
Mannital	80	PEG 4000	1.25×10^{-3}

Sources of chemicals

Polyethylene glycols (PEGs) with average molecular weights of 400, 600, and 1000 D were obtained from BDH, U.K. ^{14}C-PEG 4000 was purchased from Amersham International, U.K. Unless otherwise stated all other chemicals used were of analytical grade and all solvents for HPLC analyses were HPLC grade.

Analytical methods

High-performance liquid chromatography (HPLC) has been used to separate and quantitate the individual oligomers of a broad spectrum of PEGs (Tagesson and Sjodahl, 1984). The individual PEGs in perfusate samples were separated using a 5 μm reverse-phase column (Spherisorb, C8, 250×4.6 mm) with 42% methanol as eluent and analyzed with refractive index detection (Model 410 differential refractometer; Waters and Associates, Milford, MA). The refractometer was programmed to maintain a constant temperature of 30°C. Each perfusate sample was subjected to a direct assay without extraction. Assay standards were prepared by spiking known quantities of PEG 400, 600, and 1000 into isotonic perfusate solution. A calibration curve for each oligomer was obtained by linear regression analysis of peak height against the known total polymer concentration. The peak height for each oligomer in the unknown sample was calculated and converted to the relative amount of PEG present using the regression equation. Perfusate samples were dissolved in 4 ml of scintillation fluid (Opti Phase 'Hisafe' II; LKB Scintillation Products, U.K.) in polythene vials prior to counting ^{14}C activity in liquid scintillation spectrophotometer (1218 RackBeta Instrument, LKB Wallac, Helsinki, Finland).

Calculations

Percentage absorption of each oligomer was assessed by decreases in the concentrations of oligomers in the reservoir, with correction of any water movement by ^{14}C-PEG 4000 activity. A control experiment without the intestine indicated that there was no loss of compound during 3 hr period of the study. As a reasonable approximation loss of each oligomer from the reservoir occurred monoex-

ponentially. Apparent intestinal permeability per unit length was calculated in order to determine the intrinsic absorption potential of PEGs in ileum of the small intestine. A simple relationship relating the first-order absorption rate constant (k) to permeability coefficient was used to calculate the apparent permeability (P_{app}) of each oligomer, as follows.

$$P_{app}\ (cm\ sec^{-1}) = \frac{k \cdot V}{A} \quad (1)$$

where V is the volume of the reservoir and A is the estimated diffusional area of intestinal lumen.

RESULTS

Fig. 1 illustrates ileal absorption of PEG oligomers (330-1122 D) after recirculation for 3 hr in perfused rats. The percent absorbed of the PEGs over the 3 hr period decreased with increasing molecular weight. Absorption ranged from 27% (MW 330) to 11.6% (MW 1122) in the ileum. Net water absorption during the 3 hr period of the study was 1.3±0.42 ml in the ileum. The absorption was only 5.4 % of the total volume of the reservoir.

Table II shows the apparent permeability of PEG oligomers in perfused rats. Corrected for the length of ileum used in the study, over the molecular weight range 330 to 1122 D, the apparent permeability (P_{app}) of PEG ranged from $3.2\pm0.06\times10^{-5}$ cm/sec (mean± SEM, n=7) to $0.1\pm0.02\times10^{-5}$ cm/sec.

A logarithmic plot of the apparent permeability against molecular weight is shown in Fig. 2. The apparent permeabilities of PEGs are inversely proportional to $MW^{2.4}$ in ileum.

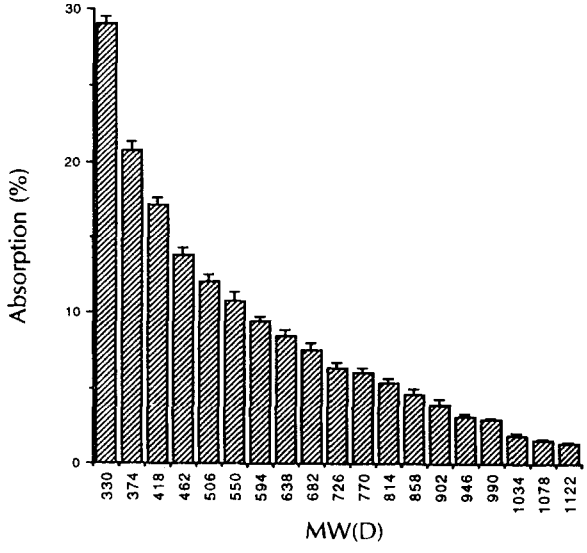

Fig. 1. Ileal absorption of PEG oligomers (330-1122 D) after recirculation for 3 hr in perfused rats. Values are means± SEM for seven rats

DISCUSSION

The intestinal epithelium has a dual role. The epithelium regulates the rate of absorption of nutrients and at the same time provides a barrier to the permeation of larger and potentially harmful compounds. This selective ability of the intestinal epithelium is often referred to as "intestinal permeability". Altered intestinal permeability may be of importance in the pathogenesis and pathophysiology of various diseases. In general terms permeability refers to the degree of penetration of the intestinal barrier by medium and large water-soluble molecules.

A variety of probes such as mannitol, rhamnose, cellobiose, lactulose, and Cr-ethylenediaminetetraacetic acid have been used to assess the permeability of

Table II. Apparent ileal permeability of PEG oligomers in perfused rats

MW	Ileum ($cm/sec\times10^{-5}$)
330	3.16±0.07[a]
374	2.21±0.12
418	1.51±0.07
462	1.25±0.10
506	1.17±0.06
550	0.99±0.06
638	0.93±0.04
682	0.82±0.05
726	0.73±0.04
770	0.68±0.04
814	0.57±0.03
858	0.47±0.04
902	0.39±0.04
946	0.34±0.03
990	0.29±0.01
1034	0.17±0.01
1078	0.15±0.01
1122	0.12±0.01

[a]Values are means±SEM for seven rats.

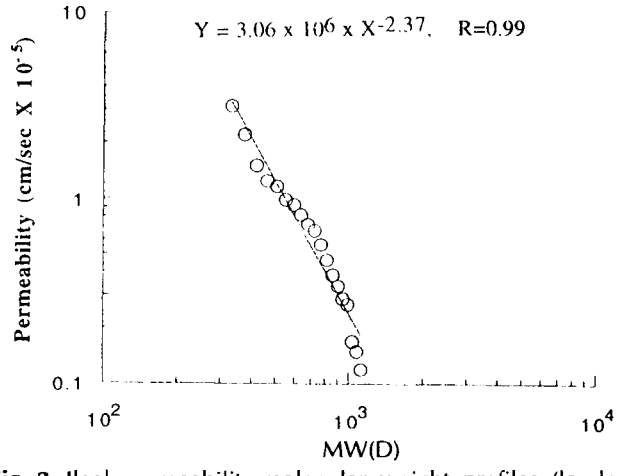

Fig. 2. Ileal permeability-molecular weight profiles (log-log plot) for PEG oligomers (330-1122 D) in perfused rats

the intestine (Peeters, 1994; Fordtran, 1965). Chadwick and his colleagues (1977a; b) introduced PEG 400 as an ideal probe for measuring intestinal permeability. Polyethylene glycol 400 is a mixture of at least nine water-soluble homologous compounds, with MW ranging from 242 to 594. Recent studies have shown that intestinal absorption of higher molecular weight oligomers occurs even up to PEG 2000, although beyond PEG 1000 very little molecular weight dependency is seen (Donovan, 1990). Most studies have involved measures of percent absorbed as a function of molecular weight. The current study is concerned with intestinal permeability of oligomers of PEG up to 1000 along the small intestine. By mixing commercial sources of PEG 400, 600 and 1000 it was possible to study simultaneously all the oligomers between 330 and 1122 D. Good resolution of each oligomer was achieved by HPLC.

Increasingly, many small polar molecules, such as hydrochlorothiazide, cimetidine and atenolol are thought to be absorbed orally via the paracellular pathway (Riley *et al.*, 1992). In an attempt to define quantitatively the factors controlling paracellular absorption, studies have been undertaken in the perfused rat intestinal preparation as a model of events in man. It is believed that the results in rat are likely to have application to man. For example, for the above mentioned polar molecules differences in permeability were found to exist between the two species (Kim and Rowland, 1992).

As a reasonable approximation loss of each oligomer from the reservoir occurred monoexponentially (Data not shown). Based on this observation, the value of the first-order absorption rate constant (k) was calculated. In this study, assuming that the segment is a distended uniform cylinder, the radius of the jejunum was 0.17 cm. This corresponded to internal surface area of 21 cm^2 for ileum. Substituting this information into Equation 1 allowed the estimation of the apparent permeability.

The results in this study (Table II and Fig. 2) demonstrated the absence of a cut-off phenomenon over the molecular weight range studied suggesting that a similar mechanism of permeation exists for both small and large molecules with movement through aqueous channels between cells. Loehry *et al* (1973) observed a linear relationship between log MW and log clearance across rabbit small intestinal mucosa for "probes" such as urea, creatinine, uric acid, and vitamin B_{12} whose MWs range from 60 to 33,000. Generally, it is assumed that intestinal permeability is inversely proportional to MW ($P \sim 1/M$) (Czaky, 1987). The ileal permeability obtained in this study was inversely proportional to $MW^{2.4}$ ($P \sim 1/M^{2.4}$). The greater exponential of MW obtained with different-sized PEGs reflects that the penetration of PEGs is more sensitive to MW change.

It was the first time to observe this relationship ($P \sim 1/M^{2.4}$) between MW and permeability using different-sized PEGs (MW range 330 to 1122 D). It can not be said at present that the relationship ($P \sim 1/M^{2.4}$) between molecular weight and intestinal permeability of polyethylene glycol is applied to all other compounds unless those experiments for other compounds are undertaken. It is probable that this relationship is general to all other compounds having similar ranges of MW/size to that of the marker compound (e.g. PEG) in health. So integrity of the gut mucosa can be predicted using the ideal marker (e.g. PEG). However, some investigators reported that intestinal permeability was better correlated with the smallest cross-sectional diameter of the probes than with molecular weight (Hollander *et al.*, 1988; Ma *et al.*, 1990). It is also possible that even some compounds with the same MW as PEG oligomers (reference marker) used in this study behave quite differently. Therefore the intestinal permeability for other compounds should be further investigated in health and disease.

The perfusate medium (Table I) containing mannitol was chosen to minimize the net movement of water across the small intestine. This objective was achieved, with only a 5% net absorption of water occurring over the 3 hr period of study. Addition of glucose has been known to cause greater water absorption in intestine (Rainbird *et al.*, 1984). Nonetheless it is important to correct concentration in the reservoir for water movement when calculating rate of solute absorption, especially for poorly absorbed compounds. We used ^{14}C-PEG 4000 as a nonabsorbable marker for fluid transport.

Permeability changes of PEG 400 have been seen in patients with inflammatory bowel diseases (Sanderson, *et al.*, 1987; Jenkins, *et al.*, 1986). Because the ileum can be involved in inflammatory bowel disease, PEGs permeation of perfused ileal segments of rats were studied. Menzies *et al* (1979) observed abnormally increased intestinal permeability to sugars (lactulose) in patients with villous atrophy. They suggested that this might be due to leakiness of the abnormal mucosa to larger polar molecules. Also, many studies have shown that absorption of di- or tripeptides was increased in patients who have severely impaired small bowel function (i.e. jejunoileal bypass, pancreatic insufficiency) (Heimburger, 1990; Zaloga, 1990; Fogel, 1975).

The mechanisms that are responsible for the increase in intestinal permeability in disorders such as Crohn's disease are not understood at present. Because probes such as PEGs are used clinically for permeability assessment, it is important to know which barriers and what mechanisms normally regulate in-

testinal penetration of such probe molecules and which routes and mechanisms control their intestinal transport. The basic information about the characteristics of a different-sized PEGs permeation across the small intestinal epithelium will be crucial for proper interpretations of clinical studies of permeability in patients with intestinal bowel disease. Therefore, the findings from this study would provide an useful base to design future studies to assess the permeability abnormalities in various desease states.

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