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Permeability of Polyethylene Glycol in Remnant Small Bowel after Massive Intestinal Resection (41547)

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Abstract. Studies were designed to determine if permeability of adapted (remnant) small bowel mucosa to polyethylene glycol (PEG) was altered after major intestinal resection. Rats underwent 50% small bowel resection with preservation of duodenum and terminal ileum. Sham-operated animals served as controls. Two and four weeks later we cannulated the portal vein and measured mucosal permeability to luminal [^{3}H]PEG and [^{14}C]PEG in isotonic Ringer solution in remnant proximal or distal in situ closed intestinal loops. A lumen-to-portal blood gradient of at least 1000/1 persisted throughout the one-hour experimental period in both resected and sham-operated animals. Thus the adapted remnant intestinal mucosa was highly impermeable to luminal radiotracer PEG. In separate experiments 2 and 4 weeks after 70% small bowel resection or sham operation, *in vivo* segments of proximal and distal small intestinal were perfused through the lumen for one hour with hypertonic (800 mOsm) mannitol or NaCl solution containing [^{3}H]PEG. There was equal and almost total recovery of [^{3}H]PEG at the end of the experimental period in resected and control animals. The combined data of all experiments indicate that radiotracer PEG may be confidently used as a luminal water phase marker in transport studies of remnant bowel following intestinal resection.

Since its introduction in 1947 (1) polyethylene glycol (PEG), molecular weight 4000, has been used in both man and experimental animals as a nonabsorbable water phase luminal volume marker in a wide variety of *in vivo* studies of intestinal transport. It has become the accepted standard marker though chemical methods of assay have remained laborious (2–5). The availability of radiolabeled PEG has served to simplify its assay (5) and recent work has shown that radiotracer PEG without the presence of stable carrier PEG in the luminal solution suffices as a water phase marker (3, 6). Intestinal resection results in both morphologic and functional adaptive changes in remnant bowel (7, 8). The rate of mucosal cell turnover in remaining intestine increases and there may be a consequent functional immaturity (9–12). Increased permeability of immature neonatal mucosa to macromolecules has been amply documented (13, 14). The permeability to PEG of remnant mucosa after intestinal resection may be altered. If so, PEG would be unsuitable for use as an indicator for luminal water movement.

Two types of experiments were performed. In rats 2 and 4 weeks after major small bowel resection mucosal permeability was examined by instilling isotonic Ringer solution containing either [^{3}H]PEG or [^{14}C]PEG into ligated loops of remnant proximal or distal small bowel. Radiotracer concentration of PEG was then measured sequentially in portal blood. In other experiments total recovery of luminal [^{3}H]PEG was measured after 1 hr *in vivo* recirculation of hypertonic solution through the lumen of segments of remnant small intestine. The results show that radiolabeled PEG, molecular weight 4000, is a valid reference substrate for luminal water movement in the adapted postresected rat intestine.

Materials and Methods. Sprague–Dawley-derived male albino rats were used in all experiments. The rats were allowed free access to tap water and commercial rat chow (Wayne Lab Blox, Allied Mills, Libertyville, Ill.) and were randomly assigned for either small bowel resection or sham operation. Food but not water was withheld overnight before surgery which was carried out under intraperitoneal (ip) pentobarbital anesthesia (4 mg/100 g body weight). Rats subjected to intestinal resection underwent laparotomy and either 50 or 70%

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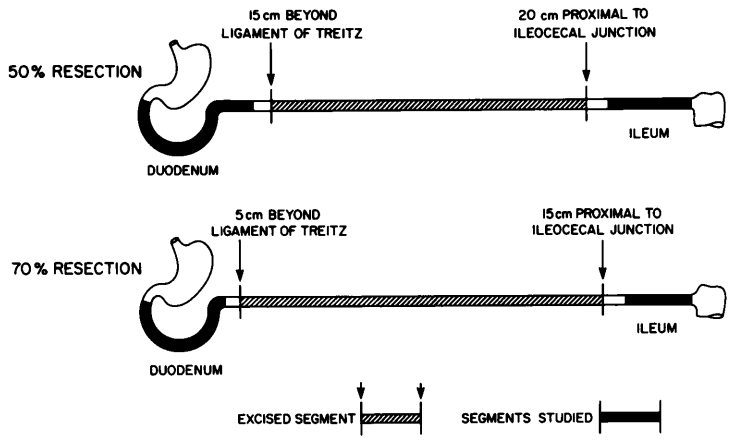

FIG. 1. Diagram showing locations of the excised segment in the two types of resected rats and the segments studied 2 or 4 weeks later. Rats with 50% small bowel resection were utilized for the closed loop experiments and one *in situ* loop was constructed in each animal. Rats with 70% small bowel resection were used for *in vivo* luminal perfusion and both proximal and distal segments were studied simultaneously in these animals. Sham-operated rats (small bowel transected at mid-small intestine followed by end-to-end reanastomosis) served as controls for each type of resection and similar segments were studied in these animals.

small bowel resection (Fig. 1). Rats from the sham-operated group underwent laparotomy and mid-small intestinal transection without removal of any intestinal tissues. In each instance continuity of the intestinal lumen was reestablished by end-to-end anastomosis. Operative details have been described (8). Either 2 or 4 weeks after surgery (ranges 13–15 days and 26–30 days) animals were anesthetized with ip urethane (0.2 g/100 g body weight) and the following experiments were performed.

Closed loop experiments. In rats with 50% intestinal resection or sham operation the portal vein was cannulated as previously described (15). An *in situ* closed intestinal loop about 20 cm long was constructed from either proximal or distal small bowel. The proximal loop began at the duodenopyloric junction and ended 10–12 cm distal to the ligament of Treitz (Fig. 1). In these animals the common bile duct was also ligated close to its entrance into the duodenum. The distal loop utilized the terminal 18–20 cm of ileum (Fig. 1). These loops will be referred to as duodenum and ileum. The loops were filled with 3.5 ml isotonic Ringer solution (16) containing about 30,000 cpm/0.1 ml tracer [^{3}H]PEG or [^{14}C]PEG (NET-405, Lot 1282-46; NET-473, Lot 1291-285, New England Nuclear Corp., Boston, Mass.). Just prior to and for five 10-min intervals after filling the intestinal loop, 0.5-ml aliquots of portal vein blood were collected (15), allowed to clot, and after centrifugation the supernatant serum was harvested for assay of radioactivity. At the end of each experiment the intestinal loop was emptied, excised, measured for length, and weighed. Aliquots, 0.1 ml of portal vein sera as well as luminal solution before and at the end of an experiment were measured for ^{3}H or ^{14}C radioactivity by standard liquid scintillation methodology. A vial containing only scintillator was used to measure background radiation with all estimations of portal vein sera radioactivity. Background radiation was subtracted from the measured radioactivity of all portal vein serum samples.

Luminal recirculation. Following anesthesia and laparotomy, the common bile duct was ligated in all rats subjected to 70% intestinal resection (Fig. 1) or sham operation. Inflow and outflow cannulas were tied into the proximal 10–12 cm and into the distal 10–12 cm of the small intestine of each animal (Fig. 1). *In vivo* luminal perfusion at 2 ml/min was then carried out. The method closely followed our prior description (8) except that the luminal perfusate was either hypertonic (800 mOsm) mannitol or hypertonic (800 mOsm) NaCl with approximately 20,000 cpm/ml

[^{3}H]PEG (NET-405, Lot 1244-104). The perfusate was recirculated for 1 hr from duodenal and ileal reservoirs each containing 25 ml solution. At the end of the perfusion period the segments were emptied by flushing with air. The lumen of each segment was then washed by passing 0.9% saline through it at a rate of 2 ml/min. The effluents from each segment were collected separately in three 10-min aliquots. Pilot studies showed that the last 10-min rinse contained only minimal radioactivity. All fluid volumes were determined by weighing. Radioactivity was measured by standard methodology in 1-ml aliquots of the perfusate before and after luminal perfusion and in 1-ml aliquots of the saline rinsing solutions. Percentage recovery of radiotracer PEG was calculated as follows: The total radioactivity present in the reservoir of each segment at the beginning of luminal perfusion (cpm/ml × initial volume) was the denominator of the fraction; the numerator was the total radioactivity in the reservoir after 1 hr recirculation (cpm/ml × final volume) plus the combined radioactivities for the three saline washes (cpm/ml × rinse volume).

All experimental data are given as mean ± SE. Statistical evaluation is based on Student's t test and P values of less than 0.05 were taken to indicate statistically significant differences.

Results. *Closed loop experiments.* Table I shows animal and intestinal data for control and resected rats 2 and 4 weeks after entry into the study. Twice as many animals were utilized in the 4 weeks group because we studied intestinal loops filled with Ringer solution and [^{14}C]PEG as well as loops filled with Ringer solution and [^{3}H]PEG. Mean body weights of each group of animals at entry into the study were identical. Two or four weeks after surgery mean body weights of the resected animals were numerically less than controls but the differences were not significant. Lengths of the loops filled with Ringer solution containing tracer PEG also did not differ. However, the weights of intestine, normalized for length, were significantly increased in both duodenum and ileum of resected rats at both study times. Table II shows concentrations of tracer PEG in the luminal solution both prior to instillation into the loops and at the conclusion of experiments 1 hr later. In duodenum, luminal tracer PEG concentrations increased significantly during all experiments, the increase in resected animals being significantly more than in the sham-operated control groups. In ileum the increase in concentration of luminal tracer PEG occurred only in resected animals. The data suggest that significant absorption of water (and presumably electrolyte) occurred in the duodenum of all animals and that there were adaptive changes of water absorption in both duodenum and ileum after resection. Although methodologies differed, the data are

TABLE I. INTESTINAL LOOPS: CONTROL AND RESECTED RATS

	2 Weeks[a]		4 Weeks[a]	
	Sham	Resected	Sham	Resected
Body weight (g)[b]				
Initial	162 ± 3 (12)	172 ± 6 (12)	168 ± 3 (24)	169 ± 3 (24)
Final	231 ± 7 (12)	226 ± 7 (12)	285 ± 5 (24)	278 ± 5 (24)
Intestinal loops				
Duodenum				
Length (cm)	19.9 ± 1.0 (6)	21.4 ± 3.1 (6)	19.1 ± 0.5 (12)	18.8 ± 0.5 (12)
Wet weight (mg/cm)[c]	72.6 ± 5.0 (6)	98.9 ± 10[d] (6)	73.9 ± 2.5 (12)	100.6 ± 2.6[d] (12)
Ileum				
Length (cm)	20.4 ± 1.6 (6)	18.2 ± 0.5 (6)	19.8 ± 0.4 (12)	19.0 ± 0.5 (12)
Wet weight (mg/cm)[c]	68.1 ± 2.0 (6)	104.2 ± 4.9[d] (6)	68.3 ± 3.0 (12)	101.5 ± 2.1[d] (12)

[a] Animals were studied 2 or 4 weeks after 50% small bowel resection or sham operation; values are mean ± SE; numbers in parentheses are the number of rats.

[b] Fasting weights immediately prior to induction of anesthesia.

[c] Full thickness intestinal tissue.

[d] Significantly different ($P < 0.05$) from corresponding data of sham-operated animals.

TABLE II. INTESTINAL LOOPS: RADIOACTIVITY OF LUMINAL SOLUTION AND PORTAL VEIN BLOOD

	2 Weeks [^{3}H]PEG[a]				4 Weeks [^{3}H]PEG[a]				4 Weeks [^{14}C]PEG[a]			
	Duodenum		Ileum		Duodenum		Ileum		Duodenum		Ileum	
	Sham	Resected	Sham	Resected	Sham	Resected	Sham	Resected	Sham	Resected	Sham	Resected
	Intestinal solution (cpm/0.1 ml)											
Initial	30,800 ± 200	33,600 ± 400	31,000 ± 100	30,900 ± 300	30,200 ± 200	30,100 ± 600	29,900 ± 400	28,700 ± 400	32,900 ± 500	32,700 ± 100	32,300 ± 300	32,500 ± 400
After 1 hr	37,000 ± 200[b]	51,700 ± 3100[b,c]	32,000 ± 600	41,000 ± 2800[b,c]	35,000 ± 400[b]	54,400 ± 100[b,c]	33,200 ± 600	42,200 ± 2400[b,c]	38,600 ± 500[b]	51,900 ± 3200[b,c]	33,600 ± 600	45,700 ± 2200[b,c]
Time (min)	Portal vein, serum (cpm/0.1 ml)[d]											
0	10 ± 6	10 ± 6	5 ± 2	7 ± 2	3 ± 1	12 ± 4	7 ± 1	10 ± 4	12 ± 4	16 ± 5	11 ± 3	12 ± 3
10	40 ± 4	28 ± 3	24 ± 5	27 ± 2	28 ± 2	31 ± 2	25 ± 3	36 ± 8	25 ± 4	28 ± 7	20 ± 4	17 ± 4
20	39 ± 7	25 ± 4	23 ± 3	26 ± 3	29 ± 2	33 ± 2	24 ± 2	31 ± 4	22 ± 2	26 ± 3	22 ± 3	22 ± 3
30	36 ± 3	32 ± 3	24 ± 4	28 ± 2	30 ± 2	34 ± 2	28 ± 3	31 ± 4	21 ± 3	21 ± 5	25 ± 4	18 ± 3
40	39 ± 2	28 ± 4	22 ± 4	26 ± 3	29 ± 1	38 ± 2	27 ± 2	31 ± 5	20 ± 2	20 ± 4	25 ± 3	16 ± 4
50	39 ± 6	33 ± 4	22 ± 5	27 ± 3	29 ± 1	40 ± 2	26 ± 2	31 ± 4	19 ± 2	21 ± 3	24 ± 3	20 ± 4
60	36 ± 4	31 ± 4	25 ± 5	27 ± 2	31 ± 1	37 ± 2	26 ± 1	30 ± 5	21 ± 3	21 ± 4	24 ± 2	23 ± 4

[a] Intestinal loops in animals studied 2 weeks after entry were filled with Ringer solution containing [^{3}H]PEG, loops in animals studied 4 weeks after entry were filled with Ringer solution containing either [^{3}H]PEG or [^{14}C]PEG; all values are mean ± SE of six observations.

[b] Significantly different ($P < 0.05$) than initial luminal tracer PEG concentration.

[c] Significantly different from corresponding data of sham-operated animals.

[d] Background radioactivity has been subtracted.

TABLE III. *In Vivo* LUMINAL PERFUSIONS WITH 800 mOsm MANNITOL

	2 Weeks[a]		4 Weeks[a]	
	Sham	Resected	Sham	Resected
Body weight (g)[b]				
Initial	156 ± 4	157 ± 4	161 ± 6	163 ± 5
Final	241 ± 9	226 ± 8	312 ± 10	294 ± 12
Intestinal segments				
Duodenum				
Mucosa, wet weight (mg/cm)	29.7 ± 2.0	37.7 ± 2.7[d]	28.7 ± 2.1	39.9 ± 2.0[d]
Water, secretion (ml/cm in 1 hr)[c]	0.150 ± 0.025	0.114 ± 0.022	0.142 ± 0.030	0.118 ± 0.014
[^{3}H]PEG, % recovery	96.1 ± 1.8	98.4 ± 1.7	98.8 ± 1.1	100.4 ± 1.1
Ileum				
Mucosa, wet weight (mg/cm)	27.3 ± 3.6	41.1 ± 3.1[d]	26.4 ± 1.8	44.7 ± 4.7[d]
Water, secretion (ml/cm in 1 hr)	0.058 ± 0.014	0.094 ± 0.017[d]	0.066 ± 0.012	0.101 ± 0.014[d]
[^{3}H]PEG, % recovery	99.9 ± 1.0	98.5 ± 1.3	98.0 ± 1.2	98.3 ± 0.7

[a] Animals were studied 2 or 4 weeks after 70% small bowel resection or sham operation; values are mean ± SE of 10 observations.
[b] Fasting weights immediately prior to induction of anesthesia.
[c] Determined from initial and final weights of perfusate reservoirs.
[d] Significantly different ($P < 0.05$) from corresponding data of sham-operated animals.

consistent with adaptations of transport activities in the postresected remnant small intestine demonstrated for several luminal substrates (7, 8, 12). Table II also shows radioactivities of portal vein sera obtained before instilling Ringer solution with tracer PEG into the intestinal loop (0 min and at 10-min intervals thereafter). In all sham and resected animals the radioactivity of portal vein blood remained very low for the whole hour compared to radioactivity in the intestinal lumen. A lumen-to-portal blood gradient of at least 1000/1 was maintained throughout this period. There were no further sequential increases of portal blood radioactivities between 10 and 60 min after the initial slight increase in the first 10 min. The data clearly indicate a very high degree of mucosal impermeability to both [^{3}H]PEG and [^{14}C]PEG.

Luminal recirculations. Table III shows data from experiments where 800 mOsm mannitol solution containing [^{3}H]PEG was recirculated for 1 hr. Body weights, both initial and final, follow the same trends as in the prior experiments. Similarly the weight of intestinal mucosa, normalized for segment lengths, also increased significantly in duodenum and ileum of resected rats at the two study times.

In the sham-operated animals at both study times net water secretion into the luminal segments was significantly greater in duodenum than ileum. In ileum after resection the increase in water secretion approximated mucosal growth. However, in duodenum where mucosal mass also increased, net water secretion did not differ from the sham-operated groups. The postresected duodenal mucosa thus exhibits a protective capacity against hyperosmolar luminal challenge not shown by remnant ileum. In spite of these different responses the mean recovery of [^{3}H]PEG at the end of the experimental period was greater than 96% in all groups of animals. Similar experiments with luminal perfusate containing 800 mOsm NaCl with [^{3}H]PEG yielded almost identical [^{3}H]PEG recoveries (data not shown). These data indicate that in the postresected remnant intestine loss of luminal [^{3}H]PEG either by transport through the mucosa or adsorption onto mucosa and tubing was minimal.

Discussion. The basic assumption underlying the use of water phase luminal volume markers in studies of intestinal transport is that concentration changes of the marker accurately reflect luminal water movement. Ideally this requires total mucosal impermeability with complete recovery of the marker from the luminal solution at the end of the experimental period. In normal intestine Carbowax 4000 or polyethylene glycol, molecular weight 4000, closely approaches this ideal (1–6).

Intestinal resection results in considerable hyperplasia of intestinal tissues. Mucosal hyperplasia occurs rapidly after enterectomy (9). It has usually been considered to have reached its maximum extent by 4 weeks after resection (10) and to persist for several months at least (17). In this study, maximum morphologic adaptation occurred 2 weeks after resection (Tables II and III), in close agreement with a prior report of maximum changes 12 days after enterectomy (11). The mucosa may exhibit a functional immaturity (9–12). The permeability of this mucosa to PEG has not been systematically examined.

Experiments with *in situ* intestinal loops clearly demonstrate that only very minimal increases in radioactivity occurred in portal blood following luminal instillation of Ringer solution containing tracer PEG (Table II). The differences between resected rats and sham-operated controls were inconsequential. The small increase in portal blood radioactivity seen in the first 10 min after instillation of substrate into the lumen was most likely the consequence of minor mucosal damage at the time of manipulation of the loops during instillations of luminal solution and tying of the final ligature. Over the next 50 min there were no further increases in portal blood radioactivities and a lumen-to-portal vein blood gradient of at least 1000/1 persisted. We deliberately used a luminal radioactivity concentration that was 15 times larger than necessary for transport studies (such as those utilized in subsequent luminal perfusions with hypertonic solutions).

Because of the very minimal mucosal permeability to both [^{3}H]PEG and [^{14}C]PEG in the closed loop experiments we decided to study radiotracer PEG recoveries after *in vivo* intestinal perfusions in animals subjected to a more extensive (70%) intestinal resection. We only examined [^{3}H]PEG recovery because there was no difference in mucosal permeability to either of the tracers (Table II). Additional stress was placed upon mucosal integrity by using strongly hypertonic luminal solutions instead of isotonic solutions. Significant increases in small intestinal mucosal permeability have been demonstrated for metabolically inert oligosaccharides during hyperosmolar luminal stimuli (18). The technique of luminal perfusion has been well established for studies of *in vivo* intestinal transport (3, 8). Following 1 hr luminal recirculation more than 96% [^{3}H]PEG was recovered from duodenum and ileum of both resected and sham-operated rats (Table III). Miller and Schedl (3) have previously reported at least 96% recovery of [^{14}C]PEG from the intestine of normal rats after 1 hr luminal perfusion with an isotonic solution.

In conclusion, the data clearly show that following enterectomy the adapted mucosa of remnant small bowel presents a formidable barrier to the absorption of radiolabeled luminal PEG and there is almost total recovery of tracer PEG after *in vivo* luminal perfusion. Therefore the material may be confidently used as a water phase marker for estimating luminal water movement in the postresected rat intestine.

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