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Aging-Associated Increase in Intestinal Permeability to Polyethylene Glycol 900

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Effect of aging on the intestinal permeability to medium size [^{3}H]polyethylene glycol (PEG 900) was examined in vivo *by gavage and* in vitro *in intestinal everted sacs of rats ranging in age from 5 to 102 weeks. Rats were gavaged with PEG 900 solution and urinary recovery of PEG 900 was measured for 6 hr in order to assess its absorption. Young rats, 5–15 weeks of age, absorbed 1–1.3% of administered PEG 900. In contrast, rats 35–102 weeks of age absorbed 1.8–2.4% of administered PEG 900 ($P < 0.05$ vs younger animals). The increased absorption of PEG 900 with aging is due to changes in intestinal permeability since the total uptake (serosal appearance + tissue uptake) of PEG 900 by jejunum, ileum, and colonic everted sacs was significantly higher ($P < 0.05$) in older rats (100 weeks) than young rats (9 ½ weeks), while urinary excretion of PEG 900 following intravenous injection was the same in the two age groups. These studies indicate that aging rats have diminished capacity to exclude larger size molecules from penetrating the intestinal mucosa. The diminished barrier functions of the small intestine with aging may allow antigenic or mutagenic compounds to reach the systemic circulation.*

Polyethylene glycol 900 (PEG 900), which is a mixture of molecules with molecular weight of 800–1000 daltons, is an ideal intestinal permeability probe because of its water solubility and because its absorption follows first-order kinetics (1). Moreover, PEG 900 is nontoxic, nondegradable by intestinal bacteria, does not undergo metabolism after absorption, and is rapidly and completely excreted in the urine after intravenous administration (1–4). Since changes in many aspects of intestinal functions have been found with aging (5–8), we examined intestinal permeability with aging using PEG 900 as a probe. We used gastric gavage or infusion of PEG 900 solution because of the rapid absorption, transport, and excretion of the molecules using this technique. Moreover, this method is simple, reproducible, and nontraumatic. This method of examining intestinal permeability has been used by other investigators to study permeability changes in celiac sprue, Crohn's disease, and a wide variety of disorders (9). In order to rule out aging-associated changes in renal clearance (10–14) of PEG 900, we measured its excretion in the urine by rats of various ages after intravenous administration. We also used everted intestinal sacs to measure possible changes in tissue uptake and serosal transport of PEG 900 with aging.

MATERIALS AND METHODS

The following materials were obtained commercially: [^{3}H]PEG 900 (New England Nuclear, Boston, Massachusetts); Ultrafluor scintillation cocktail and Fluorasol (National Diagnostic, Sommerville, New Jersey); heparinized PE-10 polyethylene catheter (Intramedic, Becton-Dickinson Co., Parsippany, New Jersey).

Methods. Male Sprague-Dawley rats were purchased as weanlings from Charles River Breeding Laboratories, Wilmington, Massachusetts, and were raised in our colony. Their body weights increased progressively with

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TABLE 1. EFFECT OF AGE ON INTESTINAL ABSORPTION OF PEG 900*

Age (weeks)	*Absorption (%)*	*No. of animals*	*Mean body weight (g)*
5	1.2 ± 0.1 —	7	157
7	1.3 ± 0.2 ($P > 0.7$)	6	315
15	1.0 ± 0.2 ($P > 0.2$)	4	419
35	1.8 ± 0.3 ($P < 0.05$)	6	630
72	2.4 ± 0.4 ($P < 0.02$)	3	756
92	2.3 ± 0.4 ($P < 0.05$)	4	807
102	1.8 ± 0.1 ($P < 0.05$)	5	950

*Values are mean ± SE. Student's *t* test was used to compare absorption value at five weeks of age (baseline) to absorption of PEG 900 by older rats.

aging (Table 1). The *in vivo* experiments were performed using fasted rats placed in individual metabolic cages, to allow the separation of urine from feces. Rats were placed in metabolic cages 24 hr prior to experimentation and then gavaged with 2 ml of [^{3}H]PEG 900 solution using a curved needle with a soft, pliable tip. The gavage solution contained 2 μCi [^{3}H]PEG 900 in 3.0 ml saline. One hour following infusion of the PEG 900, the rats were gavaged with 4 ml saline for hydration. The urine was collected for 6 hr and the volumes recorded. Three 200 μl aliquots of urine were analyzed for radioactivity.

In separate experiments, 2 μCi [^{3}H]PEG 900 was injected intravenously into the left jugular vein. The rats were then gavaged with saline, and their urine was collected and analyzed as described above.

In the *in vitro* absorption study, rats were killed by cervical dislocation, and the entire small intestine and colon removed and washed with Krebs-Ringer phosphate buffer pH 7.4 at 4° C. A 5-cm segment of the small intestine 20 cm distal to the pylorus was designated as the jejunum. A 5-cm segment of the small intestine which was 20 cm proximal to the ileocecal junction was designated as the ileum. A middle 5-cm segment of the colon was used to assess colonic permeability. Everted sacs were prepared and the serosal compartment filled with 0.4 ml of buffer (15). Sacs were incubated in Erlenmeyer flasks containing 6.0 ml of buffer and 39 nmol of total [^{3}H]PEG 900. The mucosal solution was continuously oxygenated with 95% O_2 and 5% CO_2 at 37° C in a shaking water bath (80 oscillations per min). After 20 min incubation, sacs were removed, washed, and the serosal medium drained into a scintillation vial. The sacs were then dried for 8 hr at 60° C, cut into small pieces in a scintillation vial, and 100 μl of water and 10 ml of Fluorosol (tissue solubilizer) added. After incubation at 56° C with shaking for 24 hr, the vials were cooled and counted. The *in situ* results were the mean ± SEM of at least three experiments and were expressed as a percentage of the initial gavaged or injected dose. The *in vitro* results were the mean ± SEM of at least six experiments from three rats and were expressed in nmol/5 cm segment/20 min (16).

RESULTS

Orally Administered PEG 900. In rats aged 5–15 weeks, recovery of radioactivity in urine ranged from 1 to 1.3% of administered PEG 900. Between 35 and 102 weeks of age, there was a significant ($P < 0.05$) increase in PEG 900 absorption to 1.8–2.4% (Table 1).

Intravenously Administered PEG 900. Urinary recovery of 68 ± 9% and 58 ± 3.5% of the PEG 900 was found in 9- and 100-week-old rats, respectively, over a 6 hr collection period ($P > 0.05$) (Table 2).

***In Vitro* Studies.** Table 3 shows the total uptake (serosal appearance and tissue uptake) of PEG 900 by everted sacs prepared from different regions of the intestine of 9- and 100-week-old rats. The permeability to PEG 900 was highest in the jejunum and decreased distally. At each region examined, the total uptake of PEG 900 was significantly higher ($P < 0.05$) in old rats as compared to the younger rats (Table 3). When serosal fluid accumulation of PEG 900 was measured (ng/20 min/5 cm), mean values for young (66.6, 39.8, 36.0) and old (68.1, 43.1, and 34.0) rats paralleled the total uptake into proximal jejunum distal ileum and colon as presented in Table 3. After 20 min of incubation, intestinal tissue showed an increase in mucus secretion and some blunting of jejunal villi. However, the integrity of the epithelium was mostly maintained.

DISCUSSION

Following birth, intestinal permeability to macromolecules diminishes. We examined permeability changes at the other extreme end to life—namely during aging. We examined the effect of aging on

TABLE 2. INTRAVENOUS INJECTION OF PEG 900*

Age (weeks)	*Urine recovery (%)*	*No. of animals*		*Mean body weight (g)*
9	67.9 ± 3.9%	3	($P > 0.1$)	272
100	58.2 ± 3.5%	3		669

*Values are mean ± SE. Student's *t* test was used to determine statistical significance. Urine recovery was tested in a 6-hr collection.

TABLE 3. INTESTINAL ABSORPTION OF PEG 900 *IN VITRO**

Region	*Total uptake (ng/5 cm sac/20 min)* 9 weeks	102 weeks	*Significance* (P)
Proximal jejunum	214.3 ± 10.3	280.7 ± 19.7	<0.02
Distal ileum	115.5 ± 7.1	167.7 ± 15.1	<0.01
Colon	107.0 ± 3.0	147.5 ± 11.5	<0.05

*Values are mean ± SE for total uptake after 20 min of incubation. Student's *t*-test was used to determine statistical significance.

intestinal permeability to a medium size permeability probe, PEG 900, in aging and young rats. We used PEG as a permeability probe because of its water solubility, nontoxicity, nondegradable character, and rapid excretion in the urine (1–4). We found that in young rats (5–15 weeks) absorption of PEG 900 ranged from 1 to 1.3% of administered probe while older rats (35 weeks and higher) absorbed significantly more (1.8–2.4%; $P < 0.05$). The increase in urinary recovery of the PEG 900 seen in the older rats is a function of increased intestinal permeation of PEG 900 and is not due to enhanced renal excretion of the compound since the urinary excretion of PEG 900 was the same in the two age groups following intravenous administration (Table 2). These data demonstrate clearly that changes in renal functions with aging (10–13) did not influence urinary excretion of PEG 900 and confirm the findings of Line et al, who studied the influence of aging on the urinary excretion of the smaller probe: PEG 400 (14).

We obtained further support for our conclusions when we compared the uptake and transport of PEG 900 *in vitro* by everted sacs (Table 3). Absorption was significantly greater by intestinal sacs from older animals ($P < 0.05$) in the jejunum, ileum, and colon. Moreover, absorption of PEG 900 by the jejunum was nearly twice that of the distal ileum or colon. The greater rate of PEG 900 absorption by the jejunum may be secondary to the greater surface area of the proximal jejunum when compared to the rest of the small or large bowel (17). Alternatively, the increase in jejunal absorption may be secondary to the greater permeability of the tight junctions which are more loosely structured in the proximal jejunum (18). Since we do not know whether PEG 900 is absorbed predominantly through the tight junctions or through the cell membranes, we cannot differentiate between these two possibilities at this time.

The present experiments demonstrate that aging in the rat is associated with a decrease in the barrier functions of the small and large bowel. If similar changes are found in aging persons, they may offer an explanation for the increased incidence of aging-associated disorders which can result from an increase in the intestinal absorption of environmental macromolecules such as food antigens or some large carcinogens.

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