Comparison of Olestra Absorption in Guinea Pigs with Normal and Compromised Gastrointestinal Tracts

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Female guinea pigs (12/group) were given a single dose of [14C]-olestra by gavage after consuming either 3% poligeenan in tap water (Compromised group) or just tap water (Normal group) for 5 weeks. A Sentinel group (N = 2) was given 3% poligeenan for 5 weeks. Ten sentinel animals were killed 1 day before and 10 1 day after the other animals were dosed with [14C]-olestra and their gastrointestinal tracts were examined by histology. The Compromised and Normal animals were endoscoped just before dosing with [14C]-olestra. Urine and feces were collected continuously and CO2 was collected for 7 days after dosing. The samples were analyzed for 14C and urine was also analyzed for [14C]sucrose. Animals (3/group) were killed 1, 3, 7, and 21 days after dosing, and tissues were collected and assayed for 14C. Tissue lipids were extracted, fractionated by high-pressure liquid chromatography, and analyzed for [14C]-olestra by liquid scintillation. Animals fed poligeenan showed mucosal edema, congestion, ulceration, and fibrin deposition within the distal colon and rectum. Histology revealed inflammation, epithelial degeneration, and multifocal ulceration of the cecum, distal colon, and rectum. The gastrointestinal mucosa of nonpoligeenan fed animals was normal. No [14C]-olestra was detected in liver lipids and no [14C]sucrose was found in the urine for any animal in the Normal or Compromised groups, indicating that intact olestra was not absorbed. The amount, distribution, and elimination of absorbed 14C did not differ between guinea pigs with normal and compromised gastrointestinal tracts. The poligeenan-treated animals displayed mucosal damage similar to that seen in human inflammatory bowel diseases; therefore, these results suggest that patients with inflammatory bowel conditions will not absorb olestra to any greater extent than normal healthy people. © 1997 Society of Toxicology.

Olestra is the common name for the mixture of hexa-, hepta-, and octaesters of sucrose formed with fatty acids isolated from edible oils. It has physical and organoleptic properties similar to those of traditional triglycerides (Jandaçek and Webb, 1978, Bernhardt, 1988) but is not hydrolyzed by gastric or pancreatic lipases (Mattson and Volpenhein, 1972). Olestra essentially is not absorbed intact from the gastrointestinal (GI) tract (Miller et al., 1995; Daher et al., 1996). In studies in which rats were dosed orally with radiolabeled olestra, less than 0.0008% of the dose was absorbed (Miller et al., 1995). Because of these unique properties, olestra has the potential to serve as a replacement for dietary fat, which adds no calories to the diet. Olestra has been approved by the U. S. Food and Drug Administration as a replacement for fat used in the preparation of savory snacks.

Digestion and absorption of dietary fat occurs primarily in the small intestine, beginning with the enzymatic hydrolysis of its constituent fatty acids from their glyceride backbone and subsequent absorption via the micelle-mediated absorption pathway. In normal healthy individuals, intact dietary fat does not typically reach the lower gastrointestinal tract (colon). The structure of the olestra molecule prevents this enzymatic breakdown via steric hindrance. Hence, using olestra as a fat replacement will result in olestra being present in the lower gastrointestinal tract.

Studies in the rat and the mini-pig have shown that olestra is not absorbed from a healthy GI tract (Miller et al., 1995; Daher et al., 1996). However, the possibility exists that absorption might occur if the GI epithelium is compromised. This potential requires assessment because the integrity of the lower GI epithelium (which typically is not exposed to a dietary fat) is compromised in a number of human GI diseases and conditions. Examples include ulcerative colitis, Crohn’s disease, ischemic bowel disease, infectious colitis, and acute and chronic conditions such as drug- or chemical-induced gastritis. The permeability of the intestine to large, nonmetabolizable molecules such as polyethylene glycol (PEG) and lactulose has been shown to be increased in inflammatory bowel disease (IBD) patients (Ukabam et al., 1983; Ukabam and Cooper, 1984; Bjarnason et al., 1983; Hollander et al., 1986; Stenson and Macdermott, 1991).

To determine the absorption of olestra in patients with compromised GI tracts is not practical. Therefore, a suitable animal model had to be found to establish the absorption potential. The model chosen was the poligeenan-fed guinea pig. Numerous investigators have shown that the mucosal integrity of the lower GI tract of the guinea pig is damaged when the animal is fed poligeenan, carrageenan that has been
designated by acid hydrolysis (Grasso et al., 1973; Anver and Cohen, 1976; van der Waaij et al., 1974; Marcus et al., 1988). These studies have shown that guinea pigs fed poligeenan develop lesions of the cecum, colon, and rectum. These lesions range from small focal erosions to deeper ulcers penetrating the muscularis mucosa; they are associated with infiltration of macrophages and polymorphonuclear leukocytes, edema, capillary congestion, and crypt abscesses. The lesions are similar to those observed in a variety of human inflammatory bowel disorders (Geller, 1994). Importantly, it has been shown that the GI tract of the poligeenan-fed guinea pig has increased permeability to the absorption of large molecules such as PEG (Delahunty et al., 1992).

The gastrointestinal lesions induced in guinea pigs by poligeenan are similar to those observed in patients with various inflammatory bowel conditions, and the GI tract of the poligeenan-fed guinea pig and the inflammatory bowel disease patient both have an increased permeability to the absorption of large uncharged molecules. Therefore, the poligeenan-fed guinea is a suitable model in which to assess the potential for olestra to be absorbed in patients with compromised large intestinal and rectal epithelium. The poligeenan-fed guinea pig, however, does not meet all the requirements for an acceptable animal model for determining causal factors and pathological processes involved in inflammatory bowel disease, or for evaluating therapy (Stenson, 1994).

The study presented here compares the absorption of a single oral dose of [14C]olestra in guinea pigs with normal GI tracts to the absorption in guinea pigs with GI tracts damaged by ingestion of poligeenan.

**MATERIALS AND METHODS**

**Test Groups.** The test groups and treatments are shown in Table 1. The Normal and Compromised groups were used to determine whether the absorption of olestra from a compromised GI tract is different than that from a GI tract with normal epithelium. Endoscopic examination of the animals before radiolabel dosing provided direct confirmation of poligeenan-induced damage to the GI tract. The Sentinel group was included to provide histological confirmation that poligeenan damaged the GI tract.

Because the GI tracts of the dosed animals were collected and used to measure the recovery of radiolabel, direct histological examination of the GI epithelium of those animals was precluded. Because it has been established that the GI tract of poligeenan-fed guinea pigs has an increased permeability to the absorption of large molecules, no positive control, e.g., a group fed PEG, was included in the study.

**Animals and maintenance.** Hartley guinea pigs were obtained (Charles River Laboratories, Portage, MI) at approximately 31 days of age and were acclimatized for 11 days before being placed on study. Pilot studies were conducted to determine the concentration of poligeenan and the length of treatment required to produce extensive damage to the GI tract without inducing mortality or severe morbidity. Separate studies were carried out with male and female animals; both showed the same responses to poligeenan except the females were more sensitive to poligeenan. A concentration of 3% poligeenan produced GI damage in females similar to that produced by 5% in males. The study with female guinea pigs is described in this paper. The study with males produced the same overall result.

During acclimatization the animals were housed individually in stainless steel wire-mesh cages in a room with a 12-hr light–12-hr dark cycle. Temperature (64–75°F) and humidity (30–70%) were recorded continuously. After the animals were dosed with radiolabeled olestra, they were housed individually in glass metabolism cages (Crown Glass Company, Somerville, NJ) under the same conditions of light cycle, temperature, and humidity as used during the acclimatization period. The metabolism cages were maintained at a pressure of about 1.5 mm Hg below room atmospheric pressure. Metered incoming air, 2 L/min, was drawn through two scrubbing towers. One contained Drierite (J. T. Baker Co., Phillipsburg, NJ) to remove atmospheric moisture; the other contained soda lime (Baker) to remove carbon dioxide. All aspects of the study were conducted according to the U. S. Food and Drug Administration’s Good Laboratory Practice Regulations.

**Diets.** Diet (Purina Certified Guinea Pig Chow No. 5026, Purina Mills, St. Louis, MO) was available ad libitum throughout the study, except during a 4- to 6-hr period before administration of [14C]olestra and during a 6-hr period before the animals were killed. Animals assigned to the Sentinel or Compromised group were given free access to drinking water containing 3% poligeenan (FMC Corp., Rockland, MA) and 0.5% sucrose (Sigma, St. Louis, MO), for palatability, for 35 days before dosing with [14C]olestra. The animals assigned to the Normal group were given water containing 0.5% sucrose but no poligeenan for 35 days. Normal drinking water was provided for all animals for the last 21 days of the study.

**Test material.** The radiolabeled olestra was prepared by reacting pure [14C]sucrose (10 mCi/mmol, >97.0% pure, Amersham, Arlington Heights, IL) with a mixture of C18 (palmitic), C16 (stearic), C18:1 (oleic), and C20:1 (linoleic) fatty acid methyl esters (Aldrich Chemicals, Milwaukee, WI, >97% pure) in the presence of sodium methoxide and a potassium soap catalyst (Rizzi and Taylor, 1987). The proportions of the fatty acid methyl esters were 14% C18:0, 16% C18:1, 36% C18:2, and 34% C16:0. The octa- and heptasucrose esters were separated from the reaction mixture by high-pressure liquid chromatography (HPLC) using a PrepPak 500 silica cartridge (Waters Associates, Bedford, MA). The elution solvent was argon-purged hexane containing graded amounts of ethyl ether. The purified octa- and heptasucrose were combined to produce a mixture of 82% octaester and 18% heptaoester, a typical olestra composition.

The radiolabeled olestra sample was heated in the laboratory before it was given to the guinea pigs as described in Miller et al. (1995). This was done to ensure that the olestra given to the guinea pigs was similar to that which humans would consume in snack foods prepared by frying in olestra. When olestra is heated, it undergoes the same degradation processes as those which occur when triglycerides are heated (Gardner and Sanders, 1990; Gardner et al., 1992; Henry et al., 1992). For example, polymers are produced. These polymers have the same intermolecular linkages and occur at the same levels, on a molar basis, as those found in triglycerides heated under the same conditions (Gardner et al., 1992). Thus, the amount of polymer in the sample can be used to indicate the

<table>
<thead>
<tr>
<th>Test group</th>
<th>No. of animals</th>
<th>Day 0</th>
<th>Day −35 to Day −1</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>12</td>
<td>Tap water</td>
<td>[14C]Olestra</td>
<td></td>
</tr>
<tr>
<td>Compromised</td>
<td>12</td>
<td>3% Poligeenan</td>
<td>[14C]Olestra</td>
<td></td>
</tr>
<tr>
<td>Sentinel*</td>
<td>20</td>
<td>3% Poligeenan</td>
<td>Tap water</td>
<td></td>
</tr>
</tbody>
</table>

* Ten sentinel animals were killed 1 day before the Normal and Compromised groups were dosed with [14C]labeled olestra and 10 were killed 1 day after.
TABLE 2
Schedule of Events for Studies in Which Guinea Pigs Were Dosed with 14C-Labeled Olestra

<table>
<thead>
<tr>
<th>Activity</th>
<th>Study day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed 3% poligeenan</td>
<td>X</td>
</tr>
<tr>
<td>Dose Normal and Compromised</td>
<td>X</td>
</tr>
<tr>
<td>groups with 14C-Olestra</td>
<td>X</td>
</tr>
<tr>
<td>Collect urine and feces</td>
<td>X // X</td>
</tr>
<tr>
<td>Collect CO2</td>
<td>X // X</td>
</tr>
<tr>
<td>Perform endoscopy</td>
<td>X</td>
</tr>
<tr>
<td>Necropsy</td>
<td>X X X X</td>
</tr>
</tbody>
</table>

Degree of heat abuse. Because of the small volume and the high activity of the radiolabeled test sample, it was impractical to use the sample to prepare snacks under typical commercial snack preparation conditions. Instead, it was heated in the laboratory and the polymer content was used to monitor the degree of degradation. After heating, the polymer content of the test sample was similar to that measured in nonlabeled olestra used to fry potatoes at 185°C, 8 h/day for 7 days, typical commercial frying conditions (Henry et al., 1992).

The specific activity of the radiolabeled test sample was 1.11 mCi/g, and the radiochemical purity was 99.9%. Purity was determined by injecting an aliquot onto a C18 reverse-phase HPLC column (Zorbax ODS 5 μm, DuPont, Wilmington, DE) and counting the radiolabel in the time region where olestra is known to elute. After the test sample was prepared, it was stored in hexane, under nitrogen, at 4°C until it was administered to the guinea pigs. Stability was confirmed by determining the ester distribution of the test sample before dosing.

Dosing procedures. On Day 0 of the study, animals assigned to the Normal and Compromised groups were given approximately 200 μCi of 14C-Olestra by oral gavage in a volume of 5 mL of vehicle/kg body weight. The vehicle consisted of 25% skim milk solids, 24% sucrose, 3% salt, 2% soybean oil, and 46% distilled water. The composition of the dosing vehicle was chosen to simulate the nutrient composition in a typical human meal.

Twenty-four-hour cumulative fecal samples were solubilized in Soluene 350 (Packard) at room temperature for 48–72 hr. Triplicate 1-mL aliquots were decolorized with 2-propanol and hydrogen peroxide and were counted in the same manner as the urine samples.

Expired CO2 was collected continuously for the first 7 days after dosing in absorption towers containing 1 m sodium hydroxide. A 1-g aliquot from each 24-hr collection was placed into scintillation vials containing 15 mL of scintillation cocktail (Hionic-Flour, Packard) and was counted for 5 min (Packard 4640 counter). A cocktail blank was placed in the first position of each counting program to determine background count levels. Before the test samples were counted, the status of the counter was verified by checking the photomultiplier tube normalization, counting efficiency, and background count rates. New quench curves were entered monthly, and quench correction was verified with standards.

Necropsy was performed on three animals from the Normal group and three animals from the Compromised group 1, 3, 7, and 21 days after dosing. Necropsy procedures were controlled carefully to avoid cross-contamination of samples. After an animal was killed, the carcass was washed with methylene chloride before the skin was removed, and again after the carcass was skinned and before the organs were removed. This was done to reduce the possibility of radiolabel cross-contamination of the internal organs. The GI tract was tied shut at the anus and above the cardiac sphincter before the other organs were collected to limit potential contamination of these organs by radiolabel present in the GI contents.

A control animal that had not been dosed with 14C-Olestra was killed at each scheduled necropsy, and the organs were collected as described for the dosed animals. This control animal served as an additional check on the possibility of radiolabel cross-contamination.

Samples and organs collected included blood, spleen, brain, lung, liver, kidney, heart, carcass, gallbladder (with bile), perirenal fat pads, mesenteric lymph nodes, and GI tract (with contents). After removal from the carcass, all samples and organs were immediately frozen and stored until they could be prepared for radioassay.

Blood samples were obtained by cardiac puncture before euthanasia. Approximately 0.2 g of whole blood was combusted with 0.1 mL Combusstaid (Packard), and the radiolabel content was determined. Plasma was prepared by centrifugation, and the radioactivity of a 0.1-g aliquot from each sample was determined.

Spleen, brain, lung, kidney, heart, gallbladder, perirenal fat pad, and...
mesenteric lymph nodes were homogenized in water and lyophilized. A 0.2-g aliquot of each freeze-dried tissue was combusted (306 Biological Oxidizer, Carbo-sorb, Permafluor, Packard) and counted for 5 min.

The liver was homogenized with 2:1 chloroform:methanol and filtered. The residue was dried, frozen, and lyophilized. A 0.2-g aliquot of the chloroform:methanol extract was also counted. The entire GI tract, with contents, was solubilized in Soluene 350 (Packard) by digesting at 37°C in an oven until no tissue was visible (3 to 5 days). The radiolabel content of a 1-mL aliquot of the digest was determined.

Solutions of methylene chloride used to rinse the animals and to wash the metabolism cages after necropsy were collected, evaporated, and assayed for radioactivity. The cages were washed at least twice, or until the wash solution contained an insignificant amount of radiolabel, to ensure that all feces had been removed.

Tissue lipids were extracted and separated into four fractions with size exclusion chromatography using a Waters 600E high-pressure liquid chromatograph fitted with two PL-GEL 5-μm, 500A columns, as described in Miller et al. (1995). Fractions I and II contained lipid-soluble materials having molecular weights greater than intact olestra. Olestra polymers, if present, would elute in Fraction II. Fraction III contained olestra, if any was present, and other lipids with molecular weights similar to olestra (MW = 1500–2500). Fraction IV contained lipids having molecular weights less than olestra (e.g., triglycerides). The fractions were collected directly into scintillation vials, and the remaining elution solvent was removed with a nitrogen steam. Twenty milliliters of scintillation fluid (Ultima Gold XR, Packard) and 15 1-min fractions were collected directly into scintillation vials, and the samples were counted for 10 min (Model 2500TR, Packard).

The urine samples collected within 24 hr of dosing (Day 1) were analyzed for radiolabeled sucrose, a metabolite from absorbed and metabolized [14C]olestra. A solution of 50% urine and 50% acetonitrile, to which unlabelled sucrose was added, was injected onto a μBondapak NH2 column (Waters, Milford, MA) and 15 1-min fractions were collected directly into scintillation vials. Ten milliliters of scintillation fluid (Ultima Gold HR, Packard) were added and the fractions were counted for 5 min. The elution times of fractions containing radiolabeled sucrose were established and recovery efficiency was determined by spiking control urine samples with [14C]sucrose.

Establishment of detection limits. The detection limit for radiolabel in a given sample was taken as three standard deviations above the mean background count (99% confidence limit). This value was adjusted for total sample size to determine the detection limit for radiolabel in a given organ. Detection limits were established in this manner for each tissue or sample from each animal. The individual animal detection limits for each organ or sample then were averaged to produce a group mean detection limit. Group mean detection limits ranged from 10⁻³ to 10⁻⁶ of the dose. The detection limits correspond to about 15 disintegrations per minute (dpm) per aliquot of sample counted.

RESULTS

Observations on live animals. The amounts of poligeenan consumed by animals in the Sentinel and Compromised groups were essentially equal: 4.1 and 4.0 g/kg/day, respectively. Animals in these groups exhibited several findings consistent with published observations of the effect of poligeenan on the GI tract (Grasso et al., 1973; Anver and Cohen, 1976; van der Waaij et al., 1974; Marcus et al., 1988). Diarrhea or soft stools, anogenital staining, and the presence of blood in the refuse pan were observed among animals in both groups. The animals in the Compromised group drank less water and gained less weight than the animals in the Normal group. Over the 4-week poligeenan treatment period, animals in the Sentinel and Compromised groups increased their weight by about 6%, on average, compared with about a 30% increase for the animals in the Normal group.

Endoscopy. All animals were endoscoped on Day -1 of the study. The GI tracts of all 20 poligeenan-fed animals (Compromised and Sentinel groups) showed damage to the mucosa. The damage consisted of moderate to severe edema, mild to severe congestion, and mild to moderate hemorrhage (petechia and/or ecchymosis or frank bleeding). Sixteen of the 20 animals showed mild to severe fibrin deposition along the mucosal surface; 2 had healed or healing ulcers.

One animal in the Normal group exhibited mild congestion of the intestinal wall. Figure 1 shows the endoscopic appearance of the lower GI tract of representative animals from the Normal and the Compromised group. The GI tract of the animal from the Normal group showed no evidence of mucosal damage (Fig. 1a). The GI epithelium of the animal from the Compromised group showed signs of edema, congestion, petechiation, and ecchymosis (Fig. 1b).

Gross and histopathology of sentinel animals. The GI tracts of all animals in the Sentinel group, killed either the day before or the day after the animals in the Normal and Compromised groups were dosed with [14C]olestra, showed macroscopic GI lesions. The lesions consisted of white, tan, red focal or multifocal areas of discoloration in the distal colon, the rectum, or both. The GI tracts of three control animals, not treated with poligeenan, which were examined at the same time, were normal.

A variety of microscopic lesions were found in the GI tracts of the Sentinel animals, killed either before or after the animals in the Normal and Compromised groups were dosed. Seventeen of the 20 animals had multifocal ulcers in the cecum, distal colon, and rectum. Two also had ulcers in the proximal colon. All 20 animals showed acute inflammation and epithelial degeneration of the cecum, distal colon, and rectum. Ten of the animals exhibited inflammation in the proximal colon. One also showed epithelial degeneration in the proximal colon, characterized by loss of mucosal crypts, crypt abscesses, and infiltration of inflammatory cells into the lamina propria. The degeneration was diffuse, often involving up to 100% of the cecum, distal colon, or rectal mucosa.

Figure 2a shows a photomicrograph of the distal colon of a control animal. The photomicrograph illustrates a normal mucosa (M) consisting of a delicate lamina propria (P) and a lamina epithelialis that had regular-shaped deep parallel mucosal crypts (mc) and a tall columnar surface epithelium (se) covering the luminal surface. Note the relative thickness of the mucosa and the close apposition of the muscularis mucosa (mm) to the base of the mucosal crypts.

Figure 2b shows a photomicrograph of the distal colon of an animal in the Sentinel group killed on Day 1, 2 days after
poligeenan feeding was stopped. Note the loss of surface epithelium (i.e., severe epithelial degeneration) resulting in moderate ulceration (arrows) and severe subacute inflammation that has disrupted the integrity of the muscularis mucosa (mm) and infiltrated the submucosa (S). Also note the extensive loss of mucosal crypts (mc) and the reduced depth of the mucosa (M).

**Recovery and distribution of radiolabel.** The mean recoveries of radiolabel from animals in the Normal and Compromised groups were, respectively, 102 ± 9 and 102 ± 8% of the administered dose. Table 3 shows the distribution of the recovered radiolabel, expressed as a percentage of the total recovered dose. More than 99.8% of the administered dose of radiolabel was recovered in the combination of feces, GI tract and contents, and animal and cage rinse solutions for both groups. Radiolabel in these matrices represents non-absorbed radiolabel. Averaged across all animals and necropsy days, the radiolabel recovered from these sites was
FIG. 2. (a) Photomicrograph (magnification ×550) of H&E-stained GI tissue from a control guinea pig. The distal colon of this control animal shows a normal mucosa (M) consisting of a delicate lamina propria (P) and a lamina epithelialis that demonstrates regular-shaped deep parallel mucosal crypts (mc) and a tall columnar surface epithelium (se) covering the luminal surface. Note the relative thickness of the mucosa and the close apposition of the muscularis mucosa (mm) to the base of the mucosal crypts. gc, goblet cells in mucosal crypt epithelium. (b) Photomicrograph (magnification ×275) of H&E-stained GI tissue from a poligeenan-treated guinea pig. The distal colon shows loss of surface epithelium (severe epithelial degeneration) resulting in moderate ulceration (arrows) and severe subacute inflammation that has disrupted the integrity of the muscularis mucosa (mm) and has infiltrated the submucosa (S). Note the extensive loss of the mucosal crypts (mc) and the reduced depth of the mucosa (M). se, surface epithelium. P, lamina propria. T, tunica muscularis.

99.91 ± 0.09% of the total amount recovered for animals in the Normal group and 99.89 ± 0.08% for animals in the Compromised group.

The amount of recovered radiolabel found in tissues, blood, urine, and CO₂, averaged over animals and necropsy days, was 0.11 ± 0.08% for the Compromised group and 0.09 ± 0.09% for the Normal group. Radiolabel recovered in these matrices represents absorbed radiolabel.
Lung lipids extraction of the extracted lung lipids. Lung extracts from 7 of 10 animals in the Normal group and 11 of the Compromised group had detectable radiolabel in the olestra-containing fraction. The mean detection limits for radiolabel in the olestra-containing fraction of the lipids extracted from liver or fat pads of any animal in either the Normal or the Compromised groups contained radiolabel in the fraction in which $^{14}C$-sucrose would elute. The mean detection limit for radiolabel in the sucrose-containing fraction of urine was just at the detection limits.

Radiolabel in the olestra-containing fraction of tissue lipid extracts. No radiolabel was detected in the olestra-containing fraction of the lipids extracted from liver or fat pads of any animal in either the Normal or the Compromised group. The mean detection limits for radiolabel in the olestra-containing fraction of lipids extracted from liver were, respectively $1 \times 10^{-4}$ and $1 \times 10^{-5}$% of dose for the Normal and the Compromised groups. The detection limit for radiolabel in the olestra-containing fraction of lipids extracted from fat pads was $1 \times 10^{-4}$% of dose for both groups.

For some animals in each group, radiolabel was found in the olestra-containing fraction of lipids extracted from brain, gallbladder, lymph nodes, and spleen (data not shown). These findings were not distributed consistently among animals killed at different times after dosing.

Radiolabel was recovered in urine, CO$_2$, lung, kidney, heart, and brain tissues and in blood from animals in both the Normal and the Compromised groups killed on Day 1. Urine and CO$_2$ contained greater levels of radiolabel than did any of the tissues. Radiolabel expired as CO$_2$ was expired quickly: within 72 hr, 92 and 93% of the total amount of radiolabel recovered in CO$_2$ was expired, respectively, by the animals in the Normal and the Compromised groups. No radiolabel was found in spleen, fat pads, lymph nodes, or gallbladder for animals in either group.

Radiolabel in the olestra-containing fraction of tissue lipid extracts. No radiolabel was detected in the olestra-containing fraction of the lipids extracted from liver or fat pads of any animal in either the Normal or the Compromised group. The mean detection limits for radiolabel in the olestra-containing fraction of lipids extracted from liver were, respectively $1 \times 10^{-4}$ and $1 \times 10^{-5}$% of dose for the Normal and the Compromised groups. The detection limit for radiolabel in the olestra-containing fraction of lipids extracted from fat pads was $1 \times 10^{-4}$% of dose for both groups.

For some animals in each group, radiolabel was found in the olestra-containing fraction of lipids extracted from brain, gallbladder, lymph nodes, and spleen (data not shown). These findings were not distributed consistently among animals killed at different times after dosing.

Lipids extracted from lung tissue from all animals in both the Normal and Compromised groups had radiolabel in three or more of the four lipid fractions.

Four of 10 kidney lipid extracts from animals in the Normal group and 7 of 11 kidney lipid extracts from animals in the Compromised group had radiolabel in the olestra-containing fraction. Mean amounts ranged from $<0.4 \times 10^{-4}$ to $<7.7 \times 10^{-5}$% of the dose and were greater in the Normal group for all necropsy days except Day 7 (Table 4). These levels were at most 1 order of magnitude above the detection limits.

Five of 10 heart lipid extracts from animals in the Normal group and 6 of 11 from animals in the Compromised group had radiolabel in the olestra-containing fraction. The mean amounts ranged from $<0.6 \times 10^{-5}$ to $<39.2 \times 10^{-5}$% of the dose and were greater in the Compromised group for necropsy Days 1, 3, and 7 (Table 4). These levels generally were just at the detection limits.

Radiolabel in the sucrose-containing fraction of urine. None of the urine samples from animals in the Normal or Compromised groups contained radiolabel in the fraction in which $^{14}C$-sucrose would elute. The mean detection limit for radiolabel in the sucrose-containing fraction of urine was $4 \times 10^{-5}$% of the administered dose for both the Normal and the Compromised group.

**DISCUSSION**

Olestra is not hydrolyzed by intestinal lipases (Mattson and Volpenhein, 1972) and is not absorbed intact from normal GI tracts (Miller et al., 1995; Daher et al., 1996). Because persons with inflammatory bowel diseases have increased GI permeability to large molecules (Ukabam et al., 1983; Ukabam and Cooper, 1984; Bjarnason et al., 1983; Hollander et al., 1986; Stenson and Macdermott, 1991), this study examined the potential for ingested olestra to be absorbed intact by such persons. To do this, the absorption of olestra in guinea pigs with normal GI tract was compared with that in guinea pigs with GI tracts damaged by the ingestion of poligeenan.
The poligeenan-fed guinea pig is an appropriate model in which to assess the effect of damaged GI epithelium on absorption. The gastric lesions found in such guinea pigs are morphologically comparable to the breakdown in mucosal integrity seen in various human inflammatory bowel diseases such as ulcerative colitis, Crohn's disease, and ischemic bowel disease (Geller, 1994).

In this study, feeding poligeenan for 5 weeks produced damage in the GI tract of the guinea pigs similar to that reported by others (Grasso et al., 1973; Anver and Cohen, 1976; van der Waaïj et al., 1974; Marcus et al., 1988). Endoscopic examination of the lower GI tracts of the Sentinel animals (poligeenan-fed animals killed 1 day before the animals in the other groups were dosed with radiolabeled olestra) confirmed that the GI mucosa was damaged extensively. Histological examination of the animals in the Sentinel group provided additional evidence that the animals in the Compromised group dosed with radiolabeled olestra had damaged GI tracts. The Sentinel animals consumed the same amount of poligeenan as those in the Compromised group.

The poligeenan-fed guinea pig is an appropriate model in which to assess the effect of damaged GI epithelium on absorption. The gastric lesions found in such guinea pigs are morphologically comparable to the breakdown in mucosal integrity seen in various human inflammatory bowel diseases such as ulcerative colitis, Crohn's disease, and ischemic bowel disease (Geller, 1994).

In this study, feeding poligeenan for 5 weeks produced damage in the GI tract of the guinea pigs similar to that reported by others (Grasso et al., 1973; Anver and Cohen, 1976; van der Waaïj et al., 1974; Marcus et al., 1988). Endoscopic examination of the lower GI tracts of the Sentinel animals (poligeenan-fed animals killed 1 day before the animals in the other groups were dosed with radiolabeled olestra) confirmed that the GI mucosa was damaged extensively. Histological examination of the animals in the Sentinel group provided additional evidence that the animals in the Compromised group dosed with radiolabeled olestra had damaged GI tracts. The Sentinel animals consumed the same amount of poligeenan as those in the Compromised group. Macroscopic and microscopic lesions were found in Sentinel animals killed both 1 day before and 1 day after the Normal and Compromised groups were dosed; there were no differences in the amount of damage seen in the two groups of Sentinel animals. Thus, no healing occurred in animals fed poligeenan over the 1-day period in which dosing occurred. Therefore it can be assumed that histological damage observed in the Sentinel animals was also present in the animals in the Compromised group.

The absorption of olestra in guinea pigs fed poligeenan was not different from that in the animals which had not received poligeenan. Studies in rats given $^{14}$C by iv dosing showed that if olestra were absorbed intact and not metabolized, it would reside in the liver for several days after dosing (Jandacek and Holcombe, 1991). Therefore, the presence of radiolabel in the olestra-containing fraction of lipids extracted from the livers of dosed animals is a primary measure of the amount of olestra absorbed intact and not metabolized. No radiolabel was detected in the olestra-containing fraction of liver lipids for either group of animals.

If olestra were absorbed and systemically metabolized, $^{14}$C-sucrose would be secreted in the urine because olestra would be metabolized to sucrose and fatty acids, and sucrose is poorly metabolized systemically and is excreted rapidly in the urine (Weser et al., 1967; Dahlqvist and Thomson, 1963). Sucrose in the urine is unlikely to represent sucrose absorbed intact from the GI tract because sucrose is hydrolyzed extensively to glucose and fructose in the gut (Weser et al., 1967; Dahlqvist and Thomson, 1963; Michae１is and Szepesi, 1975; Nakamura and Tamura, 1972). None of the animals in the Normal or the Compromised group had detectable levels of radiolabel in the sucrose-containing fraction of urine samples collected within 24 hr of dosing.

The lack of any detectable radiolabel in these two primary measures of absorbed olestra means that the maximum amount of $^{14}$C-olestra which was absorbed was less than sum of the detection limits of the two measurements. The detection limit for radiolabel in the olestra-containing fraction of liver lipids ranged from $<1 \times 10^{-5}$ to $1 \times 10^{-4}$%. The detection limit for radiolabel in the sucrose-containing fraction of urine was $<4 \times 10^{-4}$% of the dose for both groups. Within these limitations, it can be concluded that the absorption of olestra was the same in guinea pigs with normal and with compromised GI tracts and in both groups was less than about $10^{-5}$% of the administered dose.

For those tissues which frequently contained radiolabel
in the olestra-containing fraction of the lipid extract (lung, kidney, and heart), the differences in mean amounts of radiolabel were small and not consistent between groups. Radiolabel in these tissues is unlikely to represent absorbed olestra: studies in which rats were dosed iv with radiolabeled olestra showed that none of these organs accumulated absorbed olestra (Jandacek and Holcombe, 1991; Mattson and Jandacek, 1991). Aspiration of the [14C]olestra is the most likely source of the radiolabel found in lung lipids. Radiolabeled glucose and fructose absorbed from the GI tract after hydrolysis of trace amounts of penta- and lower esters in the radiolabeled olestra are the probable sources of the radiolabel found in the lipids from kidney and heart, and scattered throughout other tissues. Lower sucrose polyesters have been shown to be hydrolyzed in the GI tract (Miller et al., 1995; Daniel et al., 1979; Shigeoka et al., 1984). Metabolism of radiolabeled glucose and fructose would cause radiolabel to be incorporated in lipids via de novo pathways and to be distributed across all tissues. This accounts for the low levels of radiolabel detected in all tissues.

Regardless of the sources of the radiolabel in the olestra-containing fractions of the tissue lipids, the lack of any meaningful differences between the two groups shows that the fate of dosed olestra was the same in animals with normal and with compromised GI tracts. Neither the number of animals that had radiolabel in the olestra-containing fraction of tissue lipids nor the amount of radiolabel in the fraction differed consistently or meaningfully between the two groups.

Results from this study showed no difference in the absorption of olestra between guinea pigs with normal GI tracts and those with compromised GI tracts. In both groups, olestra essentially was not absorbed (<10−4% of the dose). Because the damage to the GI mucosa observed with the poligeenan treatment in these guinea pigs was comparable in kind and severity to that seen in various human inflammatory bowel conditions, these results indicate that olestra is unlikely to be absorbed in persons with compromised GI tracts. These findings support the results of a recent clinical study in patients with ulcerative colitis or Crohn’s disease, which showed that olestra can be consumed safely by persons with inflammatory bowel diseases (Zorich et al., 1994).

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