Effect of Hydrophobic Detergent on Lipid Absorption in the Rat and on Lipid and Sterol Balance in the Swine

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The effects of hydrophobic detergent on fat absorption in the rat were determined under two conditions. In the first, a high dose of detergent was given in a test lipid meal to rats not previously exposed to this agent. A marked delay in digestion of triglyceride in association with malabsorption was observed. In the second, a relatively small dose of detergent was given to rats pretreated with dietary supplement of detergent. No delay of digestion or uptake was observed but absorbed, reesterified lipid was noted to accumulate in the mucosa. Morphologic studies showed abnormal collections of fat droplets in the enterocytes. Sterol and fat balance studies were done on swine on chronic dietary detergent supplement. Mild steatorrhea with excess fecal excretion of neutral sterols was observed. It is concluded that hydrophobic detergents can have an inhibitory effect on both intraluminal and intracellular events of fat absorption.

Recently we reported that nonionic hydrophobic detergents of the pluronic polyol series inhibit intestinal absorption of triglyceride and cholesterol when studied under acute conditions in the rat (1). Furthermore, when these detergents were given in small amounts in the diet over a period of four weeks, a reduction of plasma and hepatic levels of triglyceride and cholesterol was produced (1).

The present investigation was done to obtain information on the mechanism of action of these detergents on lipid absorption and metabolism. \[^{14}\text{C}\]Triolein was employed to analyze triglyceride absorption. Studies were performed to simulate the acute and chronic conditions of the original experiments (1). For the acute studies rats not previously exposed to detergent were given a relatively large amount of detergent with the \[^{14}\text{C}\]triolein test meal. The location and the state of the \[^{14}\text{C}\]lipid in various regions of the gastrointestinal tract was determined 4 hr after feeding. In the chronic study other rats pretreated with dietary detergent supplement were given a relatively small amount of detergent with the test meal and were also allowed to digest and absorb this meal over a 4-hr period.

The effects of detergent on cholesterol absorption and metabolism were investigated under chronic conditions only. This required conducting sterol balance analyses on the experimental animals. As the rat metabolizes cholesterol in a manner quite differently from the way it is metabolized in humans, it was felt that more significant information might be obtained employing another species of animals that more closely resembles man with regards to cholesterol metabolism. Thus, the swine was selected for these sterol balance studies. Additionally, at the termination of this experiment samples of...
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plasma, liver, and gallbladder bile were obtained for determination of lipid content.

In both the rat and the swine experiments morphologic studies of the small bowel were done using light and electron microscopic techniques to complement the above chemical investigations on the effects of hydrophobic detergents on lipid absorption.

MATERIALS AND METHODS

Radioactive chemicals, [4-14C]cholesterol, [G-3H]cholic acid, and carboxyl [14C]triolein, were purchased from New England Nuclear Corp., Boston, Massachusetts. Triolein was purchased from ICN Pharmaceuticals, Inc., Cleveland, Ohio. Precise thin-layer chromatography plates were obtained from Analtech, Inc., Newark, Delaware, and N-methyl-N-nitro-N-nitroso-guanidine from Aldrich Chemical Co., Milwaukee, Wisconsin. Sil-prep, used to make silyl ether derivatives of neutral sterols and Sil-prep/DMF, used to prepare silyl ethers of acidic sterol methyl esters, were purchased from Applied Science Laboratories, Inc., State College, Pennsylvania. STDHMP hydroxysteroid dehydrogenase was obtained from Worthington, Freehold, New Jersey. Columns for gas-liquid chromatography were packed with GP 3% SP-2250 on 100/200 Supelco AW-DMCS obtained from Supelco Inc., Bellefonte, Pennsylvania. The semisynthetic diet used in the studies done in rats was prepared by United States Biochemical Corp., Cleveland, Ohio. This diet was prepared from a fat-free test diet to which safflower oil and cholesterol were added to supply 10% and 1% by weight, respectively. Protein and carbohydrate were present in the diet at 18.6% and 51.4%, respectively (2). Pluronic L-81 detergent was kindly supplied BASF Wyandotte Corp., Wyandotte, Michigan. This detergent is a block copolymer with molecular weight of 2750 containing 90% hydrophobic (polyoxypropylene) and 10% hydrophilic (polyoxyethylene) components.

The composition of the high-fat, high-cholesterol diet used in the swine study has been previously reported (3). The protein and carbohydrate components supplied by whole milk powder were present at levels of 21.2% and 28.6%, respectively. Fat from the whole milk powder and added peanut oil was 26.1%. Cholesterol content was 0.36%. Animals received 480 g of this diet per day supplying 1.725 g of cholesterol. The diet contained additional chromium oxide and B-sitosterol to supply 1 g and 413 mg per day, respectively, to quantitate fecal recovery and to determine the amount of neutral sterols metabolized by intestinal bacteria to products not detected by the assay for neutral sterols as described (3). Diets given to experimental swine were as described above with the addition of Pluronic L-81, 1%.

Studies on [14C]Trioilen Absorption in Rats Exposed to Pluronic L-81. Lipid test meals were prepared on the day of use by dissolving labeled and unlabeled lipids in diethyl ether and then evaporating off the ether using a rotary evaporator. Enough radioactive and carrier triolein were used to give 538 mg of [14C]triolein (SA = 1 μCi/mmol) per rat. The test lipid meal also supplied 12 mg of unlabeld cholesterol per rat. For the acute studies a high dose of Pluronic L-81 detergent was added to the lipidether mixture prepared for experimental rats and, after thorough mixing, the ether was evaporated as described. The lipid test meal given to experimental rats was of the same composition as used for the controls except that enough Pluronic L-81 was added to provide 138 mg per rat.

For studies on the acute effects of Pluronic L-81 treatment male Sprague-Dawley rats (weighing 150-200 g) on regular rat chow were fasted overnight and then lightly anesthetized with diethyl ether and fed the lipid test meal by gavage. They were returned to their individual metabolic cages and allowed to absorb the lipid meal for 4 hr.

After completing the assigned period allowed for absorption, animals were anesthetized and exsanguinated, and the abdomen was quickly opened. The stomach, small bowel, and colon were isolated with ligatures and removed separately as described (4). The stomach and colon were removed with care to prevent leakage of luminal contents and put into separate flasks. All feces passed after receiving the test meal were added to the colonic samples. Samples were saponified, acidified, and extracted with petroleum ether as described (4). A sample of the petroleum ether phase was taken for quantitation of [14C]lipid content by scintillation spectrometry. The small bowel was opened and luminal contents flushed out with 10 ml of sodium taurocholate, 4 mM, in 0.9% saline. Small bowel luminal contents were extracted for lipid by the Folch procedure (5) as described (4). An aliquot of the chloroform phase was taken for analysis by scintillation spectrometry to quantitate recoveries of radioactive lipids. Another aliquot was subjected to thin-layer chromatography as described (6) to determine how much of the recovered [14C]lipid was present as unhydrolyzed (undigested) triolein. The small bowel mucosa was scraped off the wall of the small bowel and analyzed in the same manner as the small bowel luminal contents to quantitate recoveries of labeled lipids in the mucosa and to determine how much of the absorbed [14C]lipid had been reesterified to the triglyceride form.

The amount (percent) of the radioactive lipid absorbed was calculated by subtracting the sum of recoveries of the lipid in the stomach, small bowel lumen, and colon from the total dose given. Any lipid recovered in the small bowel mucosa was considered to have been absorbed. The amount (percent) of the radioactive lipid absorbed and transported from the small bowel mucosa into the circulation was calculated by subtracting the total amount of the lipid recovered, including that in the small bowel mucosa, from the total dose given.

For the study of the effects of chronic Pluronic L-81 detergent treatment rats were offered a semisynthetic diet containing 10% safflower oil and 1% cholesterol ad libitum. Diets given in experiments were supplemented with Pluronic L-81, 0.5%, as described (1). After being on the diets for 4 weeks animals were fasted overnight and then fed the lipid test meal by gavage.

The preparation of the test lipid meals used in this part of the investigations was as described above with the fol-