phenolic carbohydrate complexes from ruminal fluid. These more clearly defined fractions will then be used to address the hypothesis.

Registry No. CA, 621-82-9; PCA, 7400-08-0; FA, 1135-24-6; PHBA, 99-96-7; PA, 99-50-3; VA, 121-34-6; SYA, 530-57-4; PHBAL, 123-08-0; VAN, 121-33-5; SYAL, 134-96-3; cellulose, 9004-34-6; hemicellulose, 9034-32-6.

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Intestinal Absorption, Metabolism, and Nutritional Effects of Dietary Disteryl Ethers in Mice

Nikolaus Weber,* Hildegard Benning, and Erhard Schulte

Intestinal absorption and metabolism of both di([4-^{14}C]cholesteryl) ether and di([4-^{14}C]sitosteryl) ether were studied after intragastric administration to mice. Neither of the two substrates were absorbed by the cells of the intestinal mucosa. Radioactive metabolites of the two disteryl ethers were not detected in significant quantities in the various tissues of the gastrointestinal tract and the contents of small intestine. The digesta of cecum and colon as well as feces, however, were found to contain large proportions of labeled metabolites of both substrates. Ingestion (approximately 400 mg/kg of body weight per day) of dicholesteryl ether and disitosteryl ether over a period of 4 weeks did not alter significantly either feed intake or body weight and organ weights of mice. No ill effects were observed in the animals that had received diets containing the disteryl ethers.

Refining of fats and oils consists of various technical processes, such as deacidification, deodorization, and bleaching. Disteryl ethers, e.g. dicholesteryl ether (I) and disitosteryl ether (II), that are found in small proportions in commercial fats and oils, are predominantly generated by dehydration of sterols of plant and animal origin during the bleaching process (Kaufmann et al., 1970; Homberg, 1975; Smith, 1981; Kochhar, 1983).

The aim of the present study was to investigate intestinal absorption, metabolism, and nutritional effects of these artifactual sterol derivatives that are ubiquitous constituents of commercial food and feedstuffs containing

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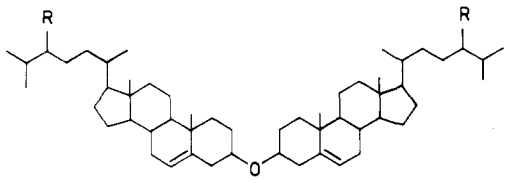

I, R = H (dicholesteryl ether)
II, R = C_2H_5 (disitosteryl ether)

refined fats and oils.

EXPERIMENTAL SECTION

Chemicals. Cholesterol and sitosterol were purchased from E. Merck, Darmstadt, FRG. [4-^{14}C]Cholesterol (2.10 GBq/mmol) and [4-^{14}C]sitosterol (2.13 GBq/mmol) were obtained from Amersham-Buchler, Braunschweig, FRG.

Preparation of Disteryl Ethers. Dicholesteryl ether and disitosteryl ether were prepared by heating the corresponding sterols with anhydrous $CuSO_4$ (Linburg and Cox, 1957). The reaction products were isolated and purified by crystallization from ethanol. Di([4-^{14}C]-cholesteryl) ether and di([4-^{14}C]disitosteryl) ether (37 MBq/mmol each) were synthesized in a similar manner from [4-^{14}C]cholesterol and [4-^{14}C]sitosterol, respectively, and purified by repeated TLC on silica gel with hexane–diethyl ether (95:5, v/v) (Kaufmann et al., 1970) and hexane–benzene (4:1, v/v) (Shapiro and Kritchevsky, 1965). The radiochemical purity of the labeled disteryl ethers was better than 98%.

Animals. Female NMRI mice (Winkelmann Versuchstierzucht, Borchen, FRG) weighing 22–27 g were used. The animals were maintained at 22 °C and 60% humidity and given feed and water ad libitum throughout the experiment.

In Vivo Administration of Di([4-^{14}C]steryl) Ethers. Radioactive dicholesteryl ether (37 MBq/mmol; 74 kBq/animal for the 2-h experiment and 148 kBq/animal for the 24-h experiment) and disitosteryl ether (37 MBq/mmol; 28 kBq/animal for the 2-h experiment) were administered by stomach tube in 0.05 mL of a vegetable oil to three animals each that had been fasted for 12 h and then fasted for 2 h further. In the 2-h experiments, distilled water, 2 mL/animal, was injected ip 1 h after administration of the radioactive substrates in order to get larger volumes of urine. The animals were maintained in metabolic cages throughout the experiment. The cages were flushed with synthetic air, and the carbon dioxide formed by oxidative degradation of feed was removed from the gas flux by absorption in triethanolamine. Aliquots of this solution were used for the determination of radioactivity by liquid scintillation counting.

Feeding Experiment. The mice were weighed and divided into three groups of eight animals each. Group 1 was given Altromin standard diet (Altromin International, Lage, FRG) having the following composition: crude protein (19%), crude fat (4%), crude fiber (6%), ash (7%), moisture (13.5%), nitrogen-free extract (50.5%). Groups 2 and 3 received the same diet containing 1.5 g of dicholesteryl ether/kg and 1.5 g of disitosteryl ether/kg, respectively, for 4 weeks. Every week, the total feed consumption and the weight gain of each group were determined. Feces of the three groups were collected over a period of 24 h every week.

Extraction Procedures. The animals of the metabolic study were sacrificed by cervical dislocation 2 or 24 h after administration of the radioactive substrates. The animals were exsanguinated by section of the aorta. Organs and tissues (stomach, intestine, liver, kidneys, heart, lungs, spleen, brain, femoral muscle tissue, and adipose tissue from the perirenal region) of three animals were quickly removed from the carcasses and weighed. Each type of tissue was pooled and homogenized in 4 mL of dichloromethane–methanol (1:2, v/v)/g of tissue. Radioactivity was determined by liquid scintillation counting in aliquots of total extracts of each tissue as well as in aliquots of pellets. The total extracts were diluted with dichloromethane and water and the phases separated by centrifugation (Bligh and Dyer, 1959). Radioactivity in both dichloromethane and water phases was determined by liquid scintillation counting. At the end of the feeding period, the animals of the nutritional experiment were killed and organs and tissues were rapidly dissected as described above. Subsequently, the total lipids were isolated as described below.

Determination of Radioactivity. Solutions were mixed with Aquasol-2 (NEN-Chemicals, Dreieich, FRG), and radioactivity was determined by liquid scintillation counting in a Tri-Carb C 2425 instrument (Packard Instruments Co., Downers Grove, IL). The distribution of radioactive fractions on thin-layer chromatograms was determined with a Berthold Automatic TLC-Linear Analyzer in combination with data acquisition system LB 500 (BF-Vertriebsgesellschaft, Wildbad, FRG).

Analysis of Radioactive Lipids. The total lipids of each tissue as well as of digesta of the various parts of the gastrointestinal tract and feces were fractionated on layers of silica gel H with chloroform–methanol–water (65:25:4, v/v/v) (Wagner et al., 1961), and the distribution of radioactivity in the various fractions was determined by scanning. The radioactively labeled fractions were each isolated and eluted from the adsorbent with water-saturated diethyl ether. The less polar lipids were further fractionated on layers of silica gel H with hexane–diethyl ether (95:5, v/v), and the distribution of radioactivity in the various lipid classes was determined. The radioactive lipid fractions were isolated as described above.

Analysis of Lipids of the Feeding Experiment. The total lipids extracted from organs and tissues of mice from groups 1–3 of the feeding experiment were isolated according to an established procedure (Bligh and Dyer, 1959). Aliquots of total lipids of the various organs and tissues were separated by TLC on silica gel H with hexane–diethyl ether (95:5, v/v). The fractions containing disteryl ethers that had been tentatively identified by cochromatography with a standard were isolated and subjected to alkaline hydrolysis. The reaction products were separated with the same solvent system. The disteryl ether fractions from various organs and tissues as well as from feces were each isolated and eluted from the adsorbent with water-saturated diethyl ether. Disteryl ethers were analyzed on a glass capillary, 20 m × 0.27 mm (i.d.), coated with 0.05-μm SE-30 (Serva, Heidelberg, FRG) at 320 °C with hydrogen (1.5 bar at column inlet; split 1:10) as carrier gas (Schulte and Weber, 1988).

Statistical Analysis. Statistical evaluation of differences between the values of body weights and organ weights of the control group and the groups that had received diets containing dicholesteryl ether and disitosteryl ether, respectively, was carried out with use of the Student's t-test for unpaired values.

RESULTS AND DISCUSSION

Intestinal Absorption and Metabolism of Radioactive Disteryl Ethers. Di([4-^{14}C]cholesteryl) ether or di([4-^{14}C]sitosteryl) ether was administered by stomach intubation to mice. Parts a and b of Figure 1 show the distribution of radioactivity from the two disteryl ethers in various organs and tissues as well as in digesta of mice

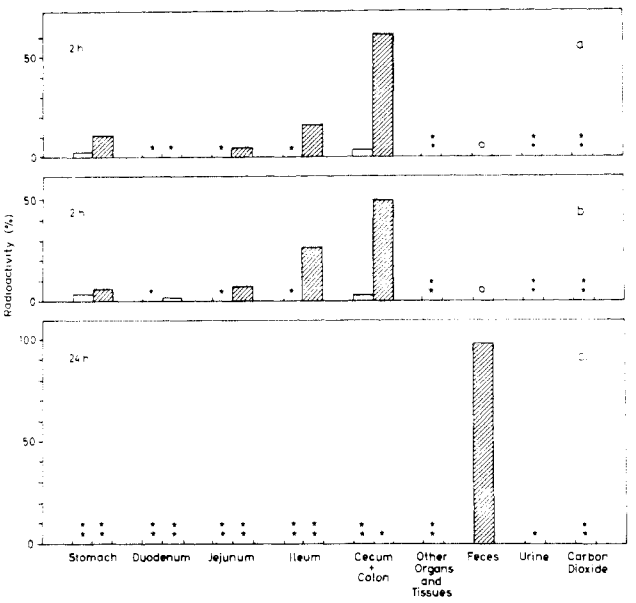

Figure 1. Distribution of radioactivity of di([4-^{14}C]steryl) ethers in various organs and tissues as well as in excretion products of mice at various times after intragastric application of the substrates: (a) di([4-^{14}C]sitosteryl) ether, 2 h after application; (b) di([4-^{14}C]cholesteryl) ether, 2 h after application; (c) di([4-^{14}C]-cholesteryl) ether, 24 h after application; left columns, radioactivity in tissues; right columns, radioactivity in the contents of stomach and intestine; asterisk, trace (<2%); two asterisks, not detected; O, not available. Average from three animals.

2 h after application of the two substrates. It is evident from these results that in all samples examined the distribution of radioactivity from dicholesteryl ether shows a close resemblance to that from disitosteryl ether. Obviously, both radioactive substrates are detected predominantly in the contents of stomach and intestine. At 2 h after stomach intubation most of the radioactivity has already passed through the intestine and is found to be concentrated in the contents of cecum and colon. It is striking that small amounts of radioactivity are extracted from intestinal tissues whereas other organs and tissues, such as kidneys, heart, lungs, spleen, muscle, brain, adipose tissue, and blood, obviously do not contain any labeled substances. Very low proportions of radioactivity (<0.1%) are detected in the liver. These findings indicate that only minor proportions of the two labeled substrates are absorbed in the intestinal mucosa—probably due to micellar solubilization of disteryl ethers—whereas the major portion of radioactive material passes without absorption through the gastrointestinal tract. The apparent absence of radioactivity in organs and tissues other than gastrointestinal tissue may be attributed to a lack of transport of disteryl ethers through the intestinal wall.

Figure 1c shows that at 24 h after intragastric application of di([4-^{14}C]cholesteryl) ether radioactivity is found almost exclusively in the feces, whereas in organs and tissues radioactivity is not detected. Carbon dioxide formed by expiration is devoid of radioactivity, as well. Traces (<0.1%) of radioactivity that are detected in urine may have resulted from contamination by feces. Obviously, even at 24 h after application of di([4-^{14}C]cholesteryl) ether the radioactivity had almost quantitatively left the alimentary canal.

In extracts from the digesta of cecum and colon as well as from feces, however, large amounts (up to 30%) of radioactive metabolites of both di([4-^{14}C]cholesteryl) ether and di([4-^{14}C]sitosteryl) ether are detected that had obviously been formed by degradation of the substrates by microorganisms of the intestinal tract. At 24 h after application of di([4-^{14}C]cholesteryl) ether the average recovery from the feces was found to be >90% of the radioactivity applied to the mice (data not shown). Moreover, the apparent absence of radioactive carbon dioxide in the expired air and of radioactive metabolites in the urine again show convincingly that the two disteryl ethers given as substrates are virtually not metabolized by cells of murine tissues.

Similar results were obtained with 3-*O*-alkyl ethers of cholesterol that are naturally occurring minor constituents of, e.g., the lipids of beef heart (Funasaki and Gilbertson, 1968). These substances resist attacks of pancreatic and intestinal enzymes. 3-*O*-Alkylcholesterols having long-chain alkyl moieties are found to be practically not absorbed by the intestinal mucosa and not metabolized by rat tissues (Paltauf, 1983). When these ether lipids are administered intravenously to rats, they remain almost unchanged over a period of 2 days (Stein et al., 1980).

Table I. Effects of Standard Diet as Well as Diets Containing Dicholesteryl Ether or Disitosteryl Ether Administered for 4 Weeks on Body Weight and Organ Weights of Mice

	body weight,[a] g ± SEM		
week	std diet[b]	dicholesteryl ether[b]	disitosteryl ether[b]
0	23.5 ± 0.3	23.3 ± 0.3	23.3 ± 0.4
1	25.2 ± 0.5	24.5 ± 0.5	24.8 ± 0.5
2	27.8 ± 0.7	26.6 ± 0.6	27.2 ± 0.5
3	29.2 ± 0.6	29.3 ± 0.4	28.8 ± 0.7
4	30.5 ± 0.6	29.8 ± 0.5	29.6 ± 0.5
	organ weight,[a] g ± SEM		
organ[c]	std diet[b]	dicholesteryl ether[b]	disitosteryl ether[b]
spleen	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.01
heart	0.15 ± 0.005	0.14 ± 0.005	0.14 ± 0.005
kidneys	0.41 ± 0.01	0.40 ± 0.01	0.40 ± 0.01
liver	1.72 ± 0.06	1.73 ± 0.05	1.77 ± 0.07

[a] All values are means ± SEM, $n = 8$; there are not statistically different values, $P > 0.2$. [b] The feed intake over 4 weeks was 6.5–7.5 g/animal per day for all experimental groups. [c] After 4 weeks.

Nutritional Effects of Disteryl Ethers. Disteryl ethers occur in industrially refined fats and oils at a level of about 5 mg/kg (Kaufmann et al., 1970). We have given dicholesteryl ether and disitosteryl ether, respectively, at a concentration as high as 1.5 g/kg standard diet to mice in order to study their nutritional effects.

Table I shows the effects of standard diet as well as diets containing dicholesteryl ether and disitosteryl ether on weight gain and feed intake of mice over a period of 4 weeks. The average feed consumption of diets containing disteryl ethers was 6.5–7.5 g/mouse per day, i.e. about 10 mg of disteryl ether/animal per day (400 mg/kg of body weight per day); the feed intake of the three groups of animals was quite similar. The results given in Table I also show that the body weight of mice receiving the dicholesteryl ether and disitosteryl ether containing diets, respectively, was statistically not different ($P > 0.2$) from the body weight of the control group.

The effects of standard diet as well as diets containing dicholesteryl ether or disitosteryl ether on weights of spleen, heart, kidneys, and liver of mice are also demonstrated in Table I. It is obvious that the weights of the various organs of the group of animals that had received the standard diet were statistically not different ($P > 0.2$) from the organ weights of animals that had been fed the disteryl ether containing diets.

The proportions of disteryl ethers in lipids from organs and tissues as well as feces of mice that had been fed diets containing dicholesteryl ether and disitosteryl ether, respectively, were determined by capillary GLC. In the lipids of tissues of the gastrointestinal tract, minor proportions (1–27 μg/g of tissue, fresh weight) of these unmetabolized ether lipids are found, whereas the lipids of other organs and tissues do not contain any disteryl ethers at all. In feces, however, major proportions (up to 3.4 mg/g of feces, dry weight) of dicholesteryl ether and disitosteryl ether are detected. These findings are in good agreement with the results that had been obtained with radioactive disteryl ethers (Figure 1).

To summarize, it was found that both di([4-^{14}C]-cholesteryl) ether and di([4-^{14}C]sitosteryl) ether are virtually not absorbed in the gastrointestinal tract of mice. Radioactivity from the two substrates is detected neither in organs and tissues outside the alimentary canal nor in urine or in carbon dioxide of the expired air. Large proportions of both unmetabolized substrates, however, are excreted with the feces. In addition, remarkable proportions of radioactive metabolites of both substrates, caused most probably by bacterial degradation, are found in digesta of cecum and colon as well as in feces.

Both dicholesteryl ether and disitosteryl ether, fed to mice at a level of 400 mg/kg of body weight for 4 weeks, do not show results different from those of the control group with respect to feed intake, weight gain, and organ weights. No ill effects are observed during this time, and no abnormalities are detected in feces (color, blood, consistency) or urine (color, blood).

These results are in good agreement with earlier findings that dicholesteryl ether neither has deleterious effects on the growth of chick heart explants in vitro (Biswas et al., 1964) nor does it induce the formation of tumors in mice and rats (Kirby, 1943; Larsen and Barrett, 1944). The influence of bile acids on the absorption of disteryl ethers will be the subject of further investigations.

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Influence of Dispersion Medium on Aroma Intensity and Headspace Concentration of Menthone and Isoamyl Acetate

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Trained judges evaluated the aroma intensities of samples containing six concentrations of methone or isoamyl acetate dispersed in distilled water, soy oil, 2, 8, and 15% NaCl, 5, 20, and 40% sucrose, or 12, 25, and 50% egg albumin. The concentration of volatile compound in the headspace was quantified by gas chromatography (GC) using a direct sampling procedure. Relative to water, oil eliminated, albumin decreased, NaCl increased, and sucrose had little effect on perceived intensity and headspace concentrations. However, differences in magnitudes of the sensory and GC responses were observed for the two compounds due to their different physical behaviors. For example, increasing concentrations of NaCl significantly increased both perceived aroma and headspace concentrations of methone, with only small increases for isoamyl acetate. Results of the sensory and GC methods were highly correlated ($r > 0.90$), but the GC was more sensitive to small changes in volatility than were the human sensors.

Foods contain many ingredients such as proteins, lipids, carbohydrates, and salts, yet little is known of the effects of these ingredients on the intensity and quality of the main flavoring. Because most food systems contain a high percentage of water, the behavior of dissolved solutes such as flavor compounds in aqueous systems is important. In dilute water solutions, flavor volatiles usually follow Henry's law (Burnett, 1963; Buttery et al., 1969, 1971; Land and Reynolds, 1981)

$$p_B = kX_B$$

where p_B is the vapor pressure of the solute, B, X_B is the

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