Effect of diosgenin on lipid metabolism in rats

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Abstract The purpose of this study was to determine whether diosgenin suppresses cholesterol absorption in rats, and to examine relevant changes in cholesterol and bile acid metabolism. Diosgenin fed with the diet for 1 week inhibited cholesterol absorption as determined by the serum isotope ratio technique, as well as by measuring in the feces the amount of unabsorbed radioactivity from orally administered [^{3}H]cholesterol. In addition, diosgenin suppressed the serum and liver uptake of radioactivity from co-administered [^{3}H]cholesterol as well as the accumulation of liver cholesterol in the cholesterol-fed rat; diosgenin was substantially more active than cholestyramine or β-sitosterol. In vitro, diosgenin had no effect on the activity of rat pancreatic esterase. Diosgenin decreased the elevated cholesterol in serum LDL and elevated cholesterol in the HDL fraction of cholesterol-fed rats; diosgenin had no effect on serum cholesterol in normocholesterolemic rats. In contrast to cholestyramine, diosgenin markedly increased neutral sterol excretion without altering bile acid excretion; in vitro, diosgenin had no effect on bile acid binding. Diosgenin treatment increased hepatic and intestinal cholesterol synthesis as well as the activity of hepatic HMG CoA reductase. This was accompanied by increased biliary concentration of cholesterol, but not of bile acids. Diosgenin had no effect on cholesterol synthesis when added to normal rat liver homogenates. It was concluded that diosgenin interferes with the absorption of cholesterol of both exogenous and endogenous origin; such interference is accompanied by derepressed, i.e., increased, rates of hepatic and intestinal cholesterol synthesis. The increased unabsorbed cholesterol together with enhanced secretion of cholesterol into bile resulted in increased excretion of neutral sterols without affecting the biliary and fecal excretion of bile acids.—**Cayen, M. N., and D. Dvornik.** Effect of diosgenin on lipid metabolism in rats. *J. Lipid Res.* 1979. **20:** 162–174.

Supplementary key words cholesterol · triglycerides · bile acids · β-sitosterol · cholestyramine · HMG CoA reductase · liver · intestine

Hypercholesterolemia is generally accepted as an independent risk factor contributing to the development of coronary heart disease (1). While controlled diet is the recommended primary treatment of hyperlipidemia (2), it is frequently more practicable to decrease the elevated lipid levels by treatment with antihyperlipidemic drugs. Hyperlipidemic states derived from elevated levels of very low density lipoproteins (VLDL) are usually quite readily controlled by drug treatment, e.g., by clofibrate (3); however, the control by drugs of elevated levels of low density lipoproteins (LDL) is not always satisfactory. At the present time, anion exchange resins are more often used to treat hypercholesterolemia (3). The resins lower plasma cholesterol by binding bile acids in the gastrointestinal tract (4), thereby increasing the rate of cholesterol catabolism (5) and/or suppressing sterol absorption (6). However, because of high dosage, the resins are not ideal for chronic treatment.

An interesting alternative treatment of hyperbetalipoproteinemia is based on the use of agents that would interfere with cholesterol absorption directly, such as β-sitosterol (7), saponins (8), and sulfaguanidine (9). In our search for such compounds, we have found that the *Solanum* glycoside tomatine inhibits cholesterol absorption in rats and markedly enhances the excretion of neutral sterols without affecting that of bile acids (10). Prompted by this finding, we were interested to determine whether the sugar moiety was required for this activity. Because of the scarcity of tomatidine, the aglycone of tomatine, we have worked with the more readily available diosgenin (**Fig. 1**), a sapogenin structurally similar to tomatidine. Diosgenin had been reported to lower serum cholesterol in chickens and rabbits fed cholesterol (11) and to decrease liver cholesterol in cholesterol-fed rats (12).

We have investigated the effect of orally administered diosgenin on cholesterol absorption in normal rats; since it was active, we have examined in some detail the effects of diosgenin on cholesterol and bile acid metabolism in the rat.[1]

During the course of our investigations, Zagoya, Laguna, and Guzman-Garcia (15) reported that, in vitro, diosgenin inhibited cholesterol uptake in an everted gut preparation and, in vivo, it enhanced

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; AUC, area under serum concentration–time curve.

[1] These studies were conducted as part of a project examining the effects of combined administration of clofibrate and inhibitors of sterol absorption on lipid metabolism (13, 14).

cholesterol synthesis and excretion without affecting the elimination of bile acids; these data were in accordance with some of our findings.

A portion of this work has appeared in abstract form (14).

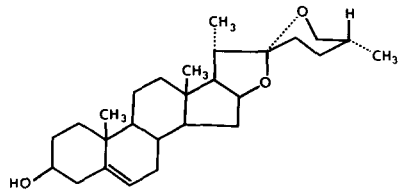

Fig. 1. Structure of diosgenin.

METHODS

Animals and diets

Male albino rats (Charles River), weighing 130–200 g, were fed powdered Purina Laboratory Chow and kept under observation for 4 days before treatment was initiated. Only animals with normal food intake and body weight gain were used. Rats were decapitated and were not fasted before killing. When used as dietary supplements, the test compounds were dissolved in chloroform, added to powdered Purina Chow or to low-cholesterol diet on metal trays, air-dried, and mixed mechanically. The low-cholesterol diet comprised 70% infant cereal (Pablum), 21% skim milk powder, 7% wheat germ, and 2% vitamin mix (Ayerst raw materials stock) (16); the concentration of cholesterol was approximately 0.02%.

Test compounds

Diosgenin was purchased from Koch-Light Laboratories (Bucks, England) and was approximately 98% pure as determined by thin-layer chromatography (TLC). Cholestyramine (Cuemid) was supplied by Merck, Sharp and Dohme of Canada (Montreal). β-Sitosterol was purchased from Sigma Chemical Co. (St. Louis, MO) and, according to the supplier's catalogue, was a "mixture of saturated and unsaturated sterols, of which approximately 60% is β-sitosterol." Cholesterol, used as food additive and as carrier in the studies on cholesterol biosynthesis, was purified via its 5,6-dibromo derivative (17) by Drs. G. Myers and T. A. Dobson from our Chemical Development and Pilot Plant.

Sodium [2-^{14}C]acetate, [24-^{14}C]cholic acid, [4-^{14}C]cholesterol, [glutaryl-3-^{14}C]-3-hydroxy-3-methylglutaryl coenzyme A and [6,7-^{3}H]cholesterol were purchased from New England Nuclear Corp. (Boston, MA). The [6,7-^{3}H]cholesterol was purified by TLC in methanol–benzene 20:80 (v/v). DL-[2-^{3}H]-Mevalono lactone, purchased from Amersham/Searle Corp. (Arlington Heights, IL), was treated with dilute methanolic KOH and neutralized to generate mevalonic acid.

Lipid levels

For determination of total cholesterol, serum (0.5 ml) was treated with 5 ml of alcoholic KOH for 1 hr at 45°C, while tissue samples or extracts were heated in alcoholic KOH for 30 min at 70–80°C. After addition of an equal volume of water, the mixture was extracted with 10 ml of *n*-hexane; an aliquot of the extract was evaporated to dryness, the residue was dissolved in 2 ml of isopropanol, and the cholesterol content was measured colorimetrically (18). Phospholipid and triglyceride levels were determined by semi-automated techniques (19, 20). Serum lipoproteins were separated into fractions of low (LDL) and high (HDL) density with dextran sulfate (21, 22); since by this procedure VLDL is coprecipitated with LDL (22), reference to LDL in this report also includes VLDL. Protein or total nitrogen was determined by the Kjeldahl digestion procedure as adapted for the autoanalyzer (23).

Cholesterol absorption

Cholesterol absorption was measured by a modification of the dual-isotope plasma ratio method of Zilversmit and Hughes (24). Rats were given 1%, 0.2%, or 0.05% diosgenin in the diet for 1 week, and were not fasted during the night prior to isotope administration. On the last day, food was withdrawn at 8:00 AM, and the isotopes were administered beginning at 2:00 PM. Animals were given per os by gavage 5 mg of [^{3}H]cholesterol (14.8 μCi/dose) in 1 ml of 2% Tween-80. The rats were then lightly anesthetized with ether and given an intravenous injection (into the external jugular vein) of 0.5 ml of a solution containing 35 μg of [4-^{14}C]cholesterol (5.3 μCi/dose); the intravenous vehicle comprised an aqueous solution containing 40% propylene glycol, 10% ethanol, 2.5% sodium benzoate, 2.5% benzoic acid, and 1.5% benzyl alcohol. The interval between the oral and intravenous doses was less than 1 min. Animals were then placed in individual metabolism cages, the feeding regimen was resumed at 8 PM and was continued for the duration of the study. Feces were collected in 24-hr intervals for 4 days and frozen pending analyses. Animals were decapitated 96 hr after isotope administration, blood was collected, and serum levels of ^{3}H and ^{14}C were measured.

The dual isotope method was validated by measuring the radioactivity content of the 4-day pooled

feces (24, 25). Since total fecal radioactivity was measured rather than that associated with fecal neutral sterols, the only potential loss would have been due to volatile bacterial degradation products of the labeled sterols; it was assumed that this loss was not only negligible but constant between groups. However, since it was found that fecal levels of ^{14}C tended to increase with the higher diosgenin doses, the amount of ^{3}H (from oral [^{3}H]cholesterol) in the feces was corrected for the presence of ^{14}C, thereby adjusting for any labeled cholesterol that had been absorbed and subsequently excreted during the 4-day period (26).

Sterols and bile acids in feces and bile

The fecal excretion of sterols and bile acids was measured as described previously (10). Daily fecal collections were lyophilized, weighed, ground to a powder, and extracted with 95% ethanol for 24 hr at 75–80°C. The extract was evaporated to dryness, and the residue was heated with 10 ml of 15% KOH in an autoclave for 4 hr at 120°C. The hydrolysate was extracted with ether to separate the neutral sterols, then it was acidified and extracted with ether to obtain the bile acids. Appropriate recovery studies were made with [^{3}H]cholesterol and [24-^{14}C]cholic acid. For determination of the total bile acid content, an aliquot of the ether extract was evaporated to dryness and the residue was taken up in 65% H_2SO_4. The solution was kept for 1 hr at 65°C and the fluorescence was measured in an Aminco-Bowman Spectrofluorometer coupled with an Electro-Instruments X-Y Recorder, Model 101B (activation wavelength, 420 nm; emission wavelength, 470 nm) (27).

The component sterols were silylated with a mixture of hexamethyldisilazane, trimethylchlorosilane, and tetrahydrofuran (28), and quantitated by gas–liquid chromatography (GLC) (Varian Model 2100 equipped with a flame ionization detector). A 6-ft column containing 1.2% SE-30 was used; temperature T_i:270°C, T_c:230°C, and T_d:270°C; the carrier gas was nitrogen, 12 ml/min at 64 psi. The retention times were as follows: coprostanol, 10.2 min; cholesterol, 17.5 min; and diosgenin, 24.5 min.

Sterol and bile acid levels in bile were analyzed in 1-ml aliquots heated with 95% ethanol, and the procedure was continued as described above for feces.

Hepatic and intestinal cholesterol synthesis

Cholesterol synthesis by rat liver homogenates was assessed by measuring the incorporation of simultaneously incubated [2-^{14}C]acetate and [^{3}H]mevalonate into neutral lipids and cholesterol (29). Liver homogenates from treated rats were incubated for 1 hr at 37°C in 0.1 M potassium phosphate buffer (pH 7.4) containing [^{14}C]acetate, [^{3}H]mevalonate, and appropriate cofactors. Neutral lipids were extracted with *n*-hexane and cholesterol was isolated and counted as its 5,6-dibromo derivative (17). In some studies, only [^{14}C]acetate incorporation into neutral lipids was measured. Although potential differences in dilution of the acetate pool may result in different absolute rates of hepatic cholesterol synthesis (30) from [^{14}C]acetate, we consider the procedure adequate to detect relative changes in the rate of cholesterol formation.

Cholesterol synthesis by rat intestinal sections was measured as described previously (31). Ilea (distal 20 cm) were flushed thoroughly with ice-cold Krebs–Ringer bicarbonate buffer, pH 7.4. Sections averaging 3 mm in length were cut with a scalpel, weighed, and incubated for 1 hr at 37°C in the buffer containing 0.1 μmol of [^{14}C]acetate (2.8 μCi). [^{3}H]Mevalonate was not used because of its limited uptake and transport to the sites of intestinal cholesterol synthesis (31, 32). Enzymatic activity was terminated by the addition of KOH pellets. Ethanol, water, and carrier cholesterol (100 mg) were added, the suspension was heated at 75–80°C for 1 hr, and the neutral lipids were extracted with *n*-hexane. Cholesterol was isolated as its 5,6-dibromo derivative as described above.

The effect of diosgenin on cholesterol synthesis was also investigated in vitro. Diosgenin, at a final concentration of 1×10^{-4} M, was incubated with 0.2 μmol of [^{14}C]acetate (5 μCi), 0.16 μmol of [^{3}H]mevalonate (0.7 μCi) and appropriate cofactors, and the incorporation into cholesterol was measured.

3-Hydroxy-3-methylglutaryl (HMG) CoA reductase

The activity of HMG CoA reductase was measured in liver microsomes of rats fed a diet containing 1% diosgenin for 1 week. After a series of preliminary studies, the procedure used was based on those described previously (33–35).

Livers were excised and immersed immediately in ice-cold saline. The livers were homogenized in 3.5 volumes of buffer (0.1 M sucrose containing 50 mM KCl and 40 mM KH_2PO_4, pH 7.2) using four strokes of a loose-fitting Potter–Elvehjem homogenizer (tolerance, 0.5 mm). Cell debris and mitochondria were sedimented by two successive centrifugations at 10,000 *g* for 15 min. The supernatant was centrifuged at 100,000 *g* for 1 hr and, after resuspension in the same volume of buffer, the microsomal pellet was recentrifuged at 100,000 *g* for 45 min. The pellet was frozen immediately (without resuspension) and subsequent operations were conducted the next day.

The microsomal pellet was resuspended in "ESM solution," the volume of which was one-fifth that used

for homogenization; ESM solution comprised 30 mM EDTA, 70 mM NaCl, and 10 mM β-mercaptoethanol, pH 6.8. An aliquot was taken for determination of protein nitrogen (23). The incubation system comprised 1 ml of ESM solution containing 30 μmol of glucose-6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, 3 μmol of $NADP^+$, 4 nmol of DL-[3-^{14}C]HMG CoA (0.03 μCi), and 5–8 mg of microsomal protein. Incubations were carried out in 25-ml beakers for 30 min at 37°C in a Dubnoff metabolic shaker. Enzymatic activity was terminated by addition of 0.2 ml of 10 N NaOH. To each sample was then added 4 mg of DL-[2-^{3}H]mevalono lactone (0.16 μCi) as internal standard, 0.2 ml of conc. HCl, and the samples were shaken at 37°C for 30 min. After addition of 1 g of anhydrous Na_2SO_4, the mixture was extracted twice with 20 ml of diethyl ether. The extracts were evaporated to dryness and the residue was dissolved in 200 μl of acetone. Twenty μl were applied to standard silica gel G TLC plates (0.25 mm layer thickness) and developed in acetone–benzene 1:1 (v/v) (36). The area containing mevalono lactone was located with short-wave ultraviolet light, the entire plate was cut into 0.5-cm bands, scraped into counting vials, digested, and counted as described in the section on radioactivity measurement. Total recovery of ^{3}H was used to calculate the enzymatic conversion of [^{14}C]HMG CoA to [^{14}C]mevalonic acid.

Cholesterol and bile acid binding in vitro

To 3 ml of cholesterol solution (ethanol–ether 1:1, v/v) (1 mg/ml) was added 3 ml of a diosgenin solution (ethanol–ether 1:1, v/v) (4 mg/ml) in a 40-ml centrifuge tube. A solution of tomatine (37) was used as positive control. The solutions were mixed, stoppered, and allowed to stand overnight at room temperature. After centrifugation, the cholesterol content in the supernatant was measured colorimetrically (18), appropriate corrections being made for the contribution of diosgenin or tomatine to the cholesterol color reaction. The difference in cholesterol content with and without binding agent indicated the amount of precipitated cholesterol.

Bile acid binding was measured by a method based on that used previously (38) Sodium taurocholate (100 mg) or sodium taurodeoxycholate (100 mg) was dissolved in 20 ml of 0.1 M potassium phosphate buffer (pH 6.2) and added to 100 mg of test compound in a 40-ml centrifuge tube. After shaking for 1 hr, an aliquot (0.1 ml) of the supernatant was added to an amount of H_2SO_4 estimated to give a 65% aqueous solution of H_2SO_4. After heating for 15 min at 60°C, the absorption at 320 nm was measured (39) using a Unicam Model SP-800 spectrophotometer. The difference between the absorbancy of bile acid in the presence and absence of the test compound was assumed to correspond to the amount of sequestered bile acid.

In addition, the capacity of diosgenin to bind cholesterol and bile acids from rat bile was determined. Bile (3 ml) was added to 5 mg of diosgenin, the mixture was shaken for 30 min and centrifuged; controls contained no diosgenin. The supernatant was analyzed for cholesterol by GLC and for bile acids fluorimetrically, as described above.

Cholesterol esterification by pancreatic tissue in vitro

Fresh pancreata from male rats were homogenized in twice the volume of 0.154 M potassium phosphate buffer (pH 6.2), centrifuged, and the supernatant was filtered through cheesecloth. The incubation conditions were based on those described previously (40, 41). The incubation medium comprised 3 ml of buffer containing 40 μmol of cholesterol, 80 μmol of sodium taurocholate, 120 μmol of oleic acid, 15 mg of bovine serum albumin (Fraction V, fatty acid-poor), and 1 μCi of [4-^{14}C]cholesterol. The medium was preincubated with diosgenin (1×10^{-2} M or 1×10^{-3} M) for 20 min at 37°C; pancreatic supernatant (2 ml) was then added and the mixture was incubated for 1 hr at 37°C in an atmosphere of 95% O_2–5% CO_2. An aliquot (4 ml) was transferred into a 50-ml volumetric flask containing hot acetone–ethanol 1:1, (v/v), heated to boiling, cooled, and made up to volume. Aliquots of 5 ml were used to precipitate free cholesterol with digitonin; the suspension was centrifuged, and the radioactivity content of the supernatant containing esterified cholesterol was measured. Cholestane-3β,5α,6β-triol (41) was used as a positive control.

Radioactivity measurement

Feces samples were lyophilized, weighed, and ground to a powder. Aliquots of serum, tissues, and feces were digested in Soluene-100 or Soluene-350 (Packard Instruments Corp.), and 15 ml of Omnifluor (5 g/l) scintillation cocktail (New England Nuclear Corp.) was added. Aqueous samples were counted in Aquasol scintillation cocktail (New England Nuclear) and others in Omnifluor (4 g/l). Gel sections from TLC plates were digested with 0.2 ml of hydrofluoric acid and 0.2 ml of water, and counted after addition of 15 ml of Aquasol.

A Packard Tri-Carb liquid scintillation counter, Model 3375, was used. The counting efficiencies for ^{14}C and ^{3}H were approximately 85% and 45%, respectively, in studies with one labeled isotope, and 50–60% and 35% in those using both ^{14}C and ^{3}H.

TABLE 1. Serum and liver radioactivity in diosgenin-treated rats fed a tracer dose (0.01% of the diet) of [6,7-^{3}H]cholesterol[a]

Group	Radioactivity Levels		Cholesterol Level
	Serum	Liver	Liver
	dpm/ml	*dpm/g*	*mg/100 g*
Control	13,610 ± 600	50,000 ± 3,330	235 ± 8.2
0.05% Diosgenin	14,270 ± 660	40,000 ± 2,390[b]	227 ± 12.1
0.2% Diosgenin	8,890 ± 340[c]	20,000 ± 1,090[c]	191 ± 14.5[b]
1.0% Diosgenin	4,220 ± 360[c]	12,300 ± 720[c]	235 ± 5.9

[a] [^{3}H]Cholesterol was mixed into the diet (0.05 μCi/g chow) and animals were treated for 1 week. Results are expressed as mean ± SEM for 10 rats/group.
[b] $P < 0.05$.
[c] $P < 0.001$.

EXPERIMENTAL

Cholesterol absorption in normal rats

Rats were fed Purina chow that contained a tracer dose of [6,7-^{3}H]cholesterol (0.01% of the diet, approximately 100,000 dpm/g chow) in addition to diosgenin supplemented in doses of 1, 0.2, and 0.05% of the diet. After 1 week, the animals were killed and radioactivity levels were measured in the liver and serum. Diosgenin decreased the serum and liver levels of radioactivity (**Table 1**) in a dose-dependent manner. Liver cholesterol levels remained unchanged. In this and in subsequent studies, diosgenin did not affect food intake or body weight gain.

The effect of diosgenin on cholesterol absorption is shown in **Table 2.** The amount of cholesterol absorbed when measured by the serum isotope ratio method was slightly higher than when measured by analysis of radioactivity in the feces. With both techniques, diosgenin fed in the diet for 1 week produced similar dose-dependent decreases in the absorption of cholesterol.

TABLE 2. Effect of diosgenin on cholesterol absorption

Group	Absorption[a]		Inhibition[b]	
	Serum Isotope Ratio	Feces	Serum Isotope Ratio	Feces
	%	%	%	%
Control	74.0 ± 2.30	63.6 ± 1.53		
1% Diosgenin	46.0 ± 5.41[d]	34.1 ± 3.45[e]	38	46
0.2% Diosgenin	55.6 ± 5.74[c]	47.0 ± 2.86[e]	25	26
0.05% Diosgenin	60.5 ± 7.62	48.8 ± 5.23[c]	18	23

[a] Percentage of orally administered [^{3}H]cholesterol absorbed, calculated from the serum isotope ratio or from the radioactivity in the pooled 4-day feces. Data are presented as mean ± SEM for 5 rats/group.
[b] Average percentage inhibition of cholesterol absorption.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] $P < 0.001$.

Subsequently, we investigated whether a single large dose of diosgenin can suppress the absorption of a tracer dose of [^{3}H]cholesterol. Rats fed Purina chow were given simultaneously a 2% Tween-80 suspension containing 1000 mg/kg of diosgenin and 2.5 μg/kg of [^{3}H]cholesterol (4 μCi); controls received [^{3}H]cholesterol only. Rats were killed at various times during the next 24 hr and serum radioactivity levels were measured. At all time intervals diosgenin caused a significant decrease in serum radioactivity levels (**Fig. 2**). This was reflected in a 33% decrease in the areas under the serum concentration–time curves (AUC) (calculated by the trapezoidal rule) in rats treated with diosgenin.

Liver cholesterol levels in the cholesterol-fed rat were also used as an index of cholesterol absorption. Rats were fed, for 1 week, chow containing 1% cholesterol in addition to diosgenin at doses ranging from 0.02% to 2% of the diet. Diosgenin produced substantial decreases in liver cholesterol levels in rats fed 1% cholesterol (**Table 3**); as little as 0.1% diosgenin sufficed to effect a statistically significant decrease. While liver phospholipids were unaltered, liver triglycerides were significantly lowered. Dietary cholesterol, either alone or in combination with diosgenin,

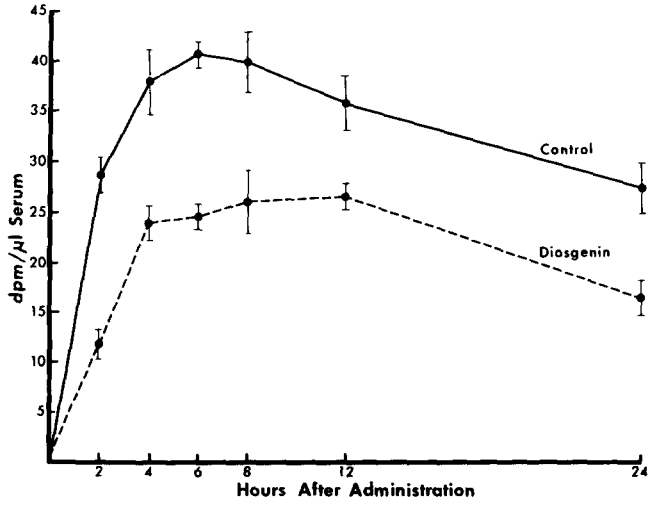

Fig. 2. Effect of a single large dose of diosgenin (1000 mg/kg) on serum ^{3}H from co-administered [^{3}H]cholesterol (4 μCi; 2.5 μg/kg). Control rats were given [^{3}H]cholesterol only. Each point is the mean (±SEM) of eight rats; all diosgenin means are significantly lower ($P < 0.01$) than the corresponding controls.

TABLE 3. Effect of diosgenin on liver lipids and adipose tissue cholesterol levels in cholesterol-fed rats[a]

Group	Liver Weight	Liver Levels			Adipose Tissue
		Cholesterol	Phospholipids	Triglycerides	Cholesterol
	g/100 g body wt	*mg/100 g*	*mg/100 g*	*mg/100 g*	*mg/100 g*
No dietary supplement	4.8 ± 0.10	244 ± 4.5	3270 ± 57	710 ± 41	62.8 ± 2.52
1% Cholesterol	4.9 ± 0.19	652 ± 56.8	3400 ± 53	1100 ± 100	68.9 ± 2.65
1% Cholesterol plus					
2% Diosgenin	5.3 ± 0.22	232 ± 10.9[d]	3450 ± 70	616 ± 68[d]	73.7 ± 1.89
1% Diosgenin	5.4 ± 0.21	261 ± 10.2[d]	3350 ± 45	607 ± 33[d]	70.4 ± 1.95
0.5% Diosgenin	5.3 ± 0.21	261 ± 7.7[d]	3730 ± 112[b]	719 ± 34[c]	69.0 ± 1.73
0.2% Diosgenin	5.1 ± 0.15	333 ± 16.4[d]	3600 ± 78[b]	784 ± 29[c]	70.0 ± 1.47
0.1% Diosgenin	5.2 ± 0.16	465 ± 43.9[b]	3430 ± 115	887 ± 68	67.1 ± 1.57
0.05% Diosgenin	5.2 ± 0.20	521 ± 30.9	3300 ± 68	1250 ± 117	64.5 ± 2.00
0.02% Diosgenin	5.0 ± 0.14	718 ± 62.8	3200 ± 60[b]	1010 ± 73	

[a] Compounds were mixed with the diet and animals were treated for 1 week. Results are expressed as mean ± SEM for 10 rats/group. Data from cholesterol + diosgenin-treated rats were compared (Student's *t* test) with those fed 1% cholesterol.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

had no effect on the concentration of cholesterol in epididymal fat pads (Table 3) (42).

The inhibitory effect of diosgenin on the liver uptake of dietary cholesterol was compared with that of β-sitosterol and cholestyramine fed to rats at doses ranging from 0.1 to 2.0% of the diet for 1 week. Diosgenin was 4–5 times more potent than β-sitosterol (**Fig. 3**). For example, 1% β-sitosterol produced the same inhibition in the liver uptake of dietary cholesterol (73%) as did 0.2% diosgenin (74% inhibition). At 1% and 2% of the diet, cholestyramine was as active as diosgenin; however, it was less active at 0.5% and inactive at 0.2% of the diet.

Sterol and bile acid excretion in rats

The effect of diosgenin on the excretion pattern of sterols and bile acids was compared with that of cholestyramine. Groups of four male rats (initial body weight, 100–120 g) were fed Purina chow supplemented with 1% diosgenin or 1% cholestyramine for 23 days. Feces were collected daily for 3 days before treatment and for the 23 days of treatment. Thus, each rat served as its own control. Sterol and bile acid extracts were prepared and analyzed. The neutral sterols were calculated from the sum of the cholesterol and coprostanol levels as measured by GLC.

The effect of diosgenin and cholestyramine on fecal sterols and bile acids is illustrated in **Figs. 4** and **5.** The data are expressed as change from pretreatment values (3-day average for each rat); during pretreatment, the animals excreted 5–10 mg/day of cholesterol + coprostanol and 10–20 mg/day of bile acids. Diosgenin markedly increased fecal sterols: up to 30 mg/day more neutral sterols were excreted by treated rats than by corresponding controls. In contrast, cholestyramine had no effect. As expected, cholestyramine increased the excretion of bile acids, while diosgenin had no effect. Thus, diosgenin increased sterol excretion without affecting the elimination of bile acids.

Serum lipids in cholesterol-fed rats

Rats were fed for 1 week a diet containing 1% cholesterol as well as diosgenin at doses ranging from 0.02% to 2% of the diet. In dose-dependent fashion, diosgenin reduced the elevated levels of serum LDL cholesterol in cholesterol-fed rats (**Table 4**); LDL phospholipid levels were also reduced, though to a lesser extent. Because of a concomitant increase in HDL

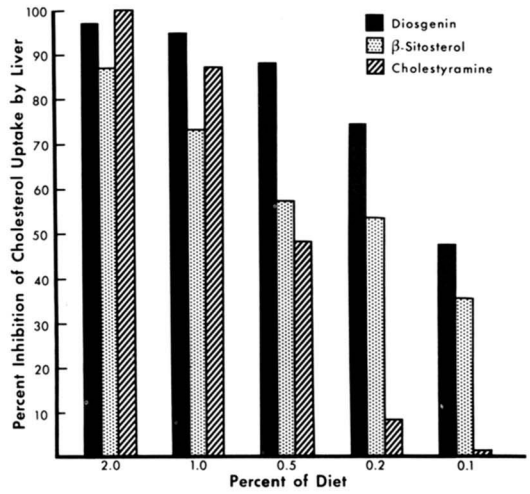

Fig. 3. Inhibitory effect of diosgenin, β-sitosterol, and cholestyramine on the liver uptake of 1% dietary cholesterol. Rats were fed diets for 1 week. Each point is the average of 10 rats.

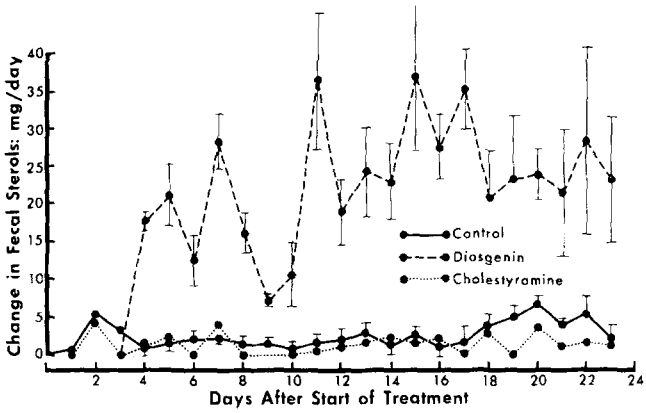

Fig. 4. Daily fecal neutral sterol excretion in diosgenin- and cholestyramine-treated rats. Diets were supplemented with 1% diosgenin or 1% cholestyramine for 23 days. Daily neutral sterol excretion was calculated from the sum of the levels of cholesterol plus coprostanol, as measured by GLC. Data are expressed as change from pretreatment (average of 3 days) levels for each rat. Each point represents the mean (±SEM) of four rats; no standard errors are given for cholestyramine-treated rats, as there was no significant difference between this group and control.

cholesterol and phospholipids, serum total cholesterol levels were not consistently decreased. Diosgenin had no effect on serum triglycerides (Table 4).

The capacity of diosgenin to affect LDL and HDL cholesterol was compared to that of β-sitosterol and cholestyramine; test compounds were administered to cholesterol-fed rats at 0.5% or 2% of the diet for 1 week. At 0.5% of the diet, diosgenin produced a significantly greater decrease in LDL cholesterol and increase in HDL cholesterol than either β-sitosterol or cholestyramine (**Fig 6**); diosgenin and cholestyramine were equipotent at 2% of the diet. The β-sitosterol-induced changes in LDL and HDL cholesterol were not statistically significant, as compared to cholesterol-fed controls (Fig. 6).

Lipid levels in normal rats

In normal rats treated for 1 week with 1% or 2% diosgenin, no detectable changes were observed in serum lipids of LDL and HDL, nor in liver lipid levels (data not shown).

Cholesterol and bile acid levels in bile

Rats with access to a diet containing 1% diosgenin were used. After 1 week, the common bile ducts were cannulated under ether anesthesia and the animals were placed in restraining cages; bile was collected for 3 hr and levels of cholesterol and bile acids were measured. In another study, the same procedure was used in rats fed a diet enriched with both 1% cholesterol and 1% diosgenin. As shown in **Table 5,** diosgenin produced a 6–9-fold increase in the concentration of cholesterol in bile of both normal and cholesterol-fed rats. Diosgenin did not affect biliary bile acid levels and had little effect on the rate of bile flow.

Hepatic cholesterol synthesis

Rats were fed for 1 week Purina chow supplemented with 0.5% diosgenin. Animals were decapitated, liver homogenates were prepared, and the incorporation of simultaneously incubated [^{14}C]acetate and [^{3}H]-mevalonate into neutral lipids and cholesterol was measured. Diosgenin produced a 23-fold increase in acetate incorporation into cholesterol (**Table 6**). The primary site of action was before mevalonate formation. A 2-fold increase in mevalonate incorporation into cholesterol was also observed. The site of this secondary effect was after the formation of neutral lipids, presumably after squalene.

Since the increase in the rate of hepatic cholesterol synthesis occurred primarily at a site before mevalonate formation, we have used the rate of acetate incorporation into neutral lipids as an indicator of diosgenin-induced derepression of cholesterol biosynthesis. Thus, at a dose as low as 0.05% of the diet, diosgenin significantly increased the rate of neutral lipid formation (**Table 7**). In addition, diosgenin, at 0.1% of the diet, increased the rate of neutral lipid formation even in rats fed 0.5% cholesterol for 1 week (Table 7). This observation provided further evidence that diosgenin interferes with cholesterol absorption.

A single dose of diosgenin (1 g/kg) had no effect on neutral lipid formation from [^{14}C]acetate by liver homogenates of rats killed 3–6 hr after dosing.

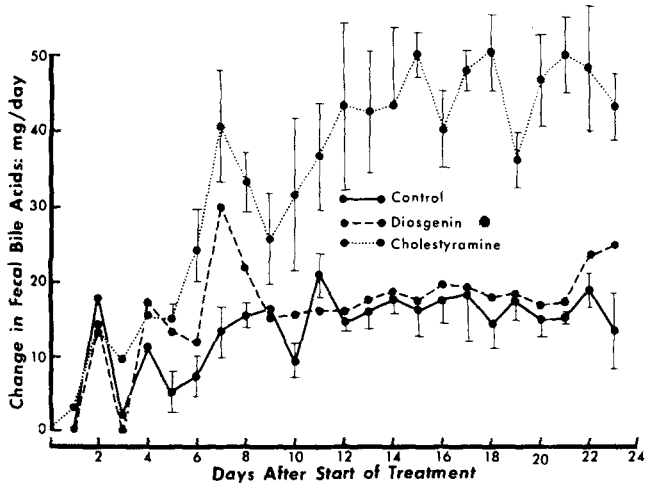

Fig. 5. Daily fecal bile acid excretion in diosgenin- and cholestyramine-treated rats. Diets were supplemented with 1% diosgenin or 1% cholestyramine for 23 days. Data are expressed as change from pretreatment (average of 3 days) levels for each rat. Each point represents the mean (±SEM) of four rats; no standard errors are given for diosgenin-treated rats, as there was no significant difference between this group and control.

TABLE 4. Effect of diosgenin on serum lipoprotein lipid levels in rats fed cholesterol[a]

Group	Serum Levels						
	Cholesterol			Phospholipids			Triglycerides
	LDL	HDL	Total	LDL	HDL	Total	Total
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
No dietary supplement	18.4 ± 1.15	32.2 ± 2.42	50.6 ± 2.81	25.2 ± 1.50	92.0 ± 6.75	117 ± 7.3	92 ± 8.3
1% Cholesterol	47.9 ± 2.85	27.2 ± 1.47	75.2 ± 3.01	42.0 ± 2.75	79.5 ± 4.25	121 ± 4.8	107 ± 12.7
1% Cholesterol plus							
2% Diosgenin	21.2 ± 1.70[d]	40.9 ± 3.46[c]	62.6 ± 2.98[c]	26.0 ± 2.50[d]	99.5 ± 5.25[c]	125 ± 5.0	87 ± 10.6
1% Diosgenin	21.4 ± 2.55[d]	47.8 ± 3.15[d]	69.2 ± 2.88	26.0 ± 2.75[d]	112.0 ± 6.50[d]	138 ± 6.0[b]	103 ± 8.7
0.5% Diosgenin	28.4 ± 1.53[d]	46.1 ± 2.08[d]	74.5 ± 3.00	36.8 ± 2.00	104.8 ± 3.00[d]	140 ± 3.8[c]	110 ± 7.7
0.2% Diosgenin	35.1 ± 1.18[d]	40.2 ± 1.71[d]	75.2 ± 1.81	40.3 ± 2.25	100.3 ± 4.00[d]	140 ± 5.0[b]	121 ± 13.3
0.1% Diosgenin	37.7 ± 1.86[c]	34.6 ± 1.28[c]	72.3 ± 2.66	36.5 ± 2.75	84.3 ± 2.00	121 ± 3.8	103 ± 15.1
0.05% Diosgenin	40.8 ± 2.19	35.8 ± 0.84[d]	76.6 ± 2.25	37.0 ± 1.00	79.3 ± 3.00	116 ± 3.5	105 ± 8.2
0.02% Diosgenin	45.3 ± 2.19	37.6 ± 2.13[d]	82.9 ± 3.54	42.5 ± 3.75	86.3 ± 5.00	129 ± 8.2	128 ± 8.0

[a] Compounds were mixed with the diet and animals were treated for 1 week. Results are expressed as mean ± SEM for 10 rats/group. Data from cholesterol + diosgenin-treated rats were compared (Student's *t* test) with those fed 1% cholesterol.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

The effect of diosgenin on cholesterol biosynthesis was also investigated in vitro. When added to normal rat liver homogenates, diosgenin (final concentration, 1×10^{-4} M) had no effect on the incorporation of [^{14}C]acetate or [^{3}H]mevalonate into cholesterol.

Intestinal cholesterol synthesis

The rate of cholesterol formation from acetate was measured in ileal sections prepared from rats fed a diet supplemented with 2% diosgenin for 1 week. Diosgenin significantly increased acetate incorporation into cholesterol (**Table 8**).

HMG CoA reductase

HMG CoA reductase activity was assessed in liver microsomes of rats given a diet supplemented with 1% diosgenin for 1 week. Diosgenin produced a 2.5-fold increase in the enzymatic reduction of DL-[3-^{14}C]HMG CoA to mevalonate (**Table 9**).

Cholesterol and bile acid binding in vitro

An ethanol–ether solution of diosgenin added to a solution of cholesterol remained clear after standing overnight at room temperature. Under the same

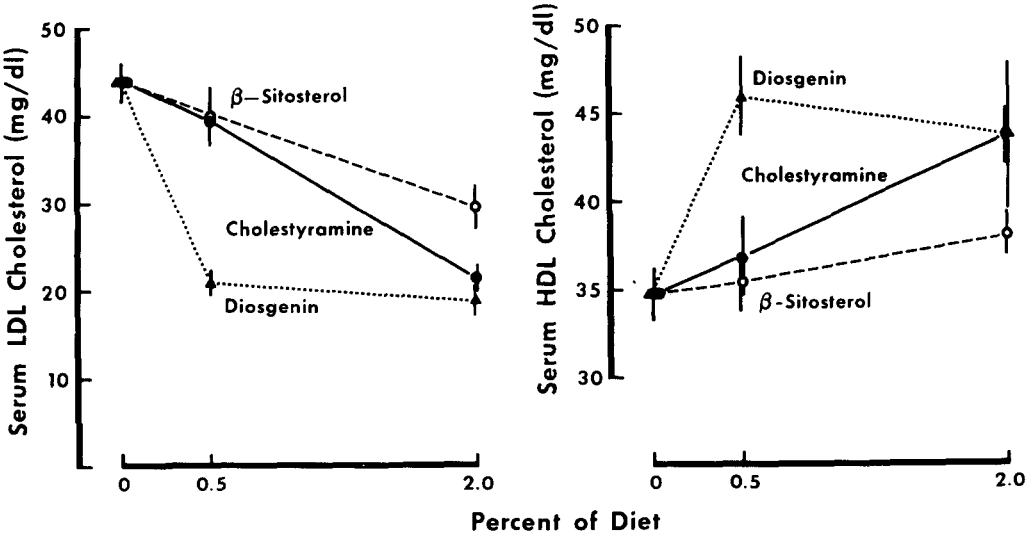

Fig. 6. Effect of diosgenin, β-sitosterol, and cholestyramine on LDL and HDL cholesterol in rats fed 1% cholesterol for 1 week. Test compounds were administered at 0.5% and 2% of the diet. Each point is the mean ± SEM for eight rats.

TABLE 5. Effect of diosgenin on cholesterol and bile acid levels in bile of normal and cholesterol-fed rats[a]

Group	Bile Volume	Concentration in Bile	
		Cholesterol	Bile Acids
	ml/3 hr	*μg/ml*	*μg/ml*
Control	3.5 ± 0.55	216 ± 33	4,190 ± 450
1% Diosgenin	4.4 ± 0.45	1,190 ± 226[c]	4,490 ± 220
1% Cholesterol	4.7 ± 0.25	124 ± 11	4,400 ± 350
1% Cholesterol + 1% Diosgenin[b]	4.1 ± 0.26	1,210 ± 270[c]	3,820 ± 310

[a] Diets were administered for 1 week. The bile ducts were then cannulated, bile was collected for 3 hr, and cholesterol and bile acids were measured in 1-ml aliquots. Results are expressed as mean ± SEM for 6 rats/group.
[b] Significance level compared with rats receiving 1% cholesterol.
[c] $P < 0.001$.

experimental conditions, a solution of tomatine precipitated 85% of the cholesterol.

The capacity of diosgenin to bind sodium taurocholate and sodium taurodeoxycholate was compared with that of cholestyramine. While cholestyramine bound both the trihydroxy- and dihydroxy-bile acids, diosgenin was virtually inactive (**Table 10**).

Diosgenin did not bind cholesterol or bile acids from rat bile in vitro.

Cholesterol esterification by pancreatic tissue in vitro

The results in **Table 11** show that, at a final concentration of 1×10^{-2} M, diosgenin had no effect on the esterification of cholesterol with oleic acid by rat pancreas. Cholestane-3β,5α,6β-triol at 1×10^{-2} M inhibited cholesterol esterification by 61%; this is similar to previously reported data (41).

DISCUSSION

These studies show that diosgenin is a potent inhibitor of cholesterol absorption in rats; the action is associated with increased rates of cholesterol synthesis, increased secretion of cholesterol into bile, and increased fecal excretion of neutral sterols.[2] Unlike ion-exchange resins, diosgenin has no effect on the excretion of bile acids.

The capacity of diosgenin to interfere with the absorption of cholesterol was shown in several ways. Similar dose-dependent decreases in cholesterol absorption were attained when analyzed by the serum isotope ratio method (24, 26) or by measuring unabsorbed radioactivity from orally administered [^{3}H]-cholesterol in a pooled feces sample (25) (Table 2). In this study, 74% of a single dose of cholesterol was absorbed in control rats when measured by the serum isotope ratio method, while a value of 64% absorption was obtained by analysis of the feces; these amounts are similar to those reported by Zilversmit and Hughes (24).

Diosgenin, at 0.1% to 2% of the diet, prevented in a dose-dependent fashion the uptake of dietary cholesterol by the liver as well as the corresponding elevation of cholesterol in serum LDL. In this model diosgenin was 4–5 times more potent than β-sitosterol and considerably more active than cholestyramine. Diosgenin also decreased serum and liver radioactivity in normal rats fed a diet containing 0.01% of ^{3}H-labeled cholesterol for 1 week; again, the effect was dose related.[3]

[2] Several studies were conducted on the metabolic disposition of diosgenin. A single oral dose of [4-^{14}C]diosgenin was very poorly absorbed in rats, dogs, and rhesus monkeys. Virtually all of the absorbed radioactivity was rapidly eliminated in the bile. Tissue distribution studies in rats showed that, of the small amounts of radioactivity absorbed, the highest concentration of drug-derived material was found in the walls of the gastrointestinal tract, liver, and adrenals. There was no detectable hepatotoxicity associated with chronic diosgenin treatment in rats and dogs. These data will be reported in detail elsewhere.

[3] It should be noted that chronic (i.e., 1 wk) treatment with diosgenin may have reduced serum and liver radioactivity, at least in part, by increasing the rate of clearance of cholesterol from the

TABLE 6. Effect of diosgenin treatment on the incorporation of [^{14}C]acetate and [^{3}H]mevalonate into cholesterol by rat liver homogenates[a]

Group	Neutral Lipids		Cholesterol	
	Acetate	Mevalonate	Acetate	Mevalonate
	dpm/mg N	*dpm/mg N*	*dpm/mg N*	*dpm/mg N*
Control	4,490 ± 690	21,790 ± 1,680	1,170 ± 300	10,150 ± 1,240
0.5% Diosgenin	37,380 ± 3,190[b] (730%)[c]	23,240 ± 690 (7%)	28,160 ± 3,950[b] (2300%)	20,260 ± 720[b] (100%)

[a] Rats were fed for 1 week Purina chow supplemented with 0.5% diosgenin. Liver homogenates were incubated simultaneously with 5.1 μCi (0.08 μmol) of sodium [2-^{14}C]acetate and 0.16 μCi (0.06 μmol) of DL-[^{3}H]mevalonic acid, and the incorporation into hexane-soluble nonsaponifiable lipids and cholesterol was measured. Results are expressed as mean ± SEM for 9 rats/group.
[b] $P < 0.001$.
[c] Values in parentheses represent percent increase over control.

TABLE 7. Effect of low doses of diosgenin on acetate incorporation into neutral lipids by liver homogenates of control and cholesterol-fed rats

Group	Neutral Lipids
	dpm ^{14}C/mg N
Control[a]	610 ± 92
0.25% Diosgenin	14,110 ± 2,070[e]
0.10% Diosgenin	2,020 ± 370[d]
0.05% Diosgenin	2,070 ± 560[c]
0.5% Cholesterol[b]	540 ± 57
0.5% Cholesterol + 0.1% Diosgenin	7,480 ± 1,090[e]

[a] Animals were treated for 1 week and decapitated. Liver homogenates were incubated with 2.0 μCi (0.03 μmol) of sodium [2-^{14}C]acetate, and the incorporation into neutral lipids was measured. Results are expressed as mean ± SEM for 9 rats/group.
[b] In a separate study, homogenates were incubated with 1.5 μCi (0.03 μmol) of sodium [2-^{14}C]acetate. Results are expressed as mean ± SEM for 9 rats/group.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] $P < 0.001$.

The pronounced increase in the rate of hepatic cholesterol synthesis provides indirect evidence that diosgenin interferes with cholesterol absorption. By inhibiting the absorption of cholesterol, diosgenin prevents exogenous cholesterol from suppressing the rate of hepatic cholesterol synthesis. As diosgenin is capable of preventing the absorption of very small amounts of dietary cholesterol (Tables 1 and 2), it is likely that the enhancement of cholesterol synthesis in normal rats is due to suppressed absorption of both dietary cholesterol normally present in Purina chow as well as of cholesterol undergoing enterohepatic circulation, i.e., that diosgenin interferes with the absorption of cholesterol regardless of its origin. Our data provide further evidence that cholesterol undergoing enterohepatic circulation contributes to the regulation of the rate of hepatic cholesterol synthesis (46).

Diosgenin increased the rate of hepatic cholesterol synthesis primarily by acting at a site preceding the formation of mevalonate, at least in part by increasing the activity of HMG CoA reductase. This is expected, since hepatic HMG CoA reductase activity is regulated by the cholesterol flux into the liver (47). Our data do not exclude the possible contribution of other enzymes, e.g., the HMG CoA condensing enzyme (48). A secondary site of action was evident after the formation of neutral lipids. We have frequently observed

body. We were unable to detect any inhibition of cholesterol absorption by measuring serum and liver ^{3}H radioactivity levels after a single low dose of diosgenin and [^{3}H]cholesterol given in dose ratios found to be effective after addition to the diet for 1 week; only with a large single oral dose (1000 mg/kg) of diosgenin and a tracer dose of [^{3}H]cholesterol was there a significant decrease in serum radioactivity levels. These differences are reminiscent of the contradictory reports on the effect of β-sitosterol on cholesterol absorption in rats. Thus, when β-sitosterol was mixed in the diet, the absorption of co-administered cholesterol was decreased (7, 43); however, when given as a single oral dose, β-sitosterol had little or no effect on cholesterol absorption (44, 45). It is likely that chronic treatment with diosgenin (or β-sitosterol) ensures adequate distribution of the agent throughout the intestinal sites of cholesterol absorption, and would therefore result in more consistent experimental data than those generated when single oral doses are administered.

TABLE 8. Intestinal cholesterol synthesis in diosgenin-treated rats[a]

Group	Neutral Lipids	Cholesterol
	dpm ^{14}C/mg N	*dpm ^{14}C/mg N*
Control	73,700 ± 14,200	4,100 ± 1,070
2% Diosgenin	164,300 ± 25,200[c] (120%)[d]	11,400 ± 2,910[b] (180%)[d]

[a] Rats were fed Purina chow supplemented with 2% diosgenin for 1 week. Ileal sections were prepared and incubated with 2.8 μCi (0.1 μmol) of [2-^{14}C]acetate, and the incorporation into neutral lipids and cholesterol was measured. Results are expressed as mean ± SEM for 8 rats/group.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] Values in parentheses represent percent increase over control.

TABLE 9. HMG CoA reductase activity in liver microsomes of diosgenin-treated rats[a]

Group	Mevalonate Synthesized	
	dpm/30 min/mg N[b]	*pmol/min/mg N*[b]
Control	15,530 ± 2,070	31.4 ± 4.2
1% Diosgenin	40,470 ± 3,300[c]	81.8 ± 6.7[c]

[a] Rats were treated for 1 week and the incorporation of DL-[3-^{14}C]HMG CoA into mevalonate by liver microsomes was measured. Results are expressed as mean ± SEM for 8 rats/group.
[b] mg N, mg of microsomal protein nitrogen.
[c] $P < 0.001$.

TABLE 10. Effect of diosgenin and cholestyramine on bile acid binding in vitro[a]

Group	Sodium Taurocholate		Sodium Taurodeoxycholate	
	Concentration	Amount Bound	Concentration	Amount Bound
	μg/0.1 ml	*%*	*μg/0.1 ml*	*%*
Control	500		500	
Diosgenin	435	13	545	0
Cholestyramine	220	56	340	32

[a] Bile salt (100 mg) was dissolved in 20 ml of 0.1 M potassium phosphate buffer (pH 6.2) and added to 100 mg of diosgenin or cholestyramine. After shaking for 1 hr and centrifugation, the bile acid content in 0.1 ml of the supernatant was measured spectrophotometrically. The difference in bile acid concentration between control and treated samples was assumed to be due to bile acid sequestered by the binding agent. Results are also expressed as percent bile acid bound by test substance and are averages of triplicate determinations.

TABLE 11. Esterification of cholesterol by rat pancreatic tissue in vitro[a]

Group	Cholesterol Ester Content	Amount Esterified	Change from Control
	dpm/incubation	%	%
Control	1,025,000	44	
1×10^{-2} M Diosgenin	1,148,000	49	+4
1×10^{-3} M Diosgenin	962,000	41	−3
1×10^{-2} M Cholestanetriol	407,000	17	−61
1×10^{-3} M Cholestanetriol	1,001,000	43	−1

[a] Compounds were incubated in supernatant obtained from homogenized rat pancreas and appropriate additives. Free cholesterol was precipitated with digitonin, and the amount of esterified cholesterol that remained in the supernatant was measured. Cholestane-3β,5α,6β-triol was used as positive control (41). Results are averages of triplicate incubations.

that agents that affect cholesterol synthesis before the formation of mevalonate also elicit a secondary effect at later stages (10, 49, 50).

At relatively high doses, diosgenin produced a moderate, though statistically significant, increase in the rate of intestinal cholesterol synthesis.[4] Since diosgenin had no apparent effect on bile acid metabolism, our finding is at variance with the concept that the rate of intestinal cholesterol synthesis is controlled only by circulating bile acids (52). We have previously found that the rate of intestinal cholesterol synthesis can be altered by changes in the amount of cholesterol absorbed. For example, dietary cholesterol is capable of suppressing intestinal cholesterol formation in cholestyramine-treated rats (49); tomatine, an agent that inhibits cholesterol absorption without affecting bile acid turnover, enhances intestinal cholesterogenesis, and it was suggested that the rate of cholesterol turnover may also contribute to the control of intestinal cholesterol synthesis (10). More recently, Shefer et al. (53) have found increased activity of intestinal HMG CoA reductase in rats fed 2% β-sitosterol, and have proposed that both bile acids and cholesterol regulate the activity of the enzyme in the intestine.

Diosgenin had no effect on serum cholesterol levels in normal rats. It is likely that the normal rat can effectively compensate for the diosgenin-induced decrease in cholesterol absorption by an increase in the rate of cholesterol biosynthesis, thus preventing a change in the level of circulating cholesterol. In addition, the diosgenin-induced increase in hepatic cholesterogenesis was accompanied by an increase in cholesterol secretion into bile, thereby precluding a rise in serum cholesterol levels. However, in rats fed cholesterol, small doses of diosgenin lowered the high cholesterol levels in LDL and increased cholesterol in HDL; higher doses of cholestyramine were required to elicit these changes.

Why diosgenin and cholestyramine increase HDL cholesterol in cholesterol-fed rats is not clear. In human hypercholesterolemia, cholestyramine therapy produces a fall in LDL cholesterol (54, 55) but no increase in HDL cholesterol (54). The cholesterol-fed rat may not be an ideal model for human hypercholesterolemia because of certain dissimilarities between rat and human lipoproteins (56) and the relative resistance of rats to induction of hypercholesterolemia by dietary cholesterol alone. In spite of these reservations, the observation that diosgenin is capable of reducing LDL and elevating HDL lipids, if operable in man, provides an ideal rationale for the control of human hypercholesterolemia (57).

Adipose tissue cholesterol levels were unaltered in rats fed cholesterol for 1 week, with or without diosgenin. In view of the report by Angel and Farkas (58) that cholesterol tends to accumulate in adipose tissue of rats fed cholesterol for 2–5 months, it is likely that our studies were too short to affect the cholesterol levels in adipose tissue.

The fundamental difference between the mode of action of diosgenin and the anion-exchange resins is in the capacity of diosgenin to increase the excretion of cholesterol without affecting that of bile acids; in contrast, the anion-exchange resins increase the excretion of bile acids without affecting the elimination of neutral sterols (59). The chow that we used in the excretion study contained 0.06% cholesterol; based on an average daily food intake of 20 g, the rats ingested approximately 12 mg of cholesterol per day. Since diosgenin increased the excretion of neutral sterols by 20–35 mg/day, the eliminated sterols must be, in part, of endogenous origin and supplied by the enhanced rate of hepatic cholesterol synthesis, followed by its increased secretion into the bile; these phenomena were observed in both normal and cholesterol-fed rats treated with diosgenin.

In order to be absorbed and transferred to the lymph, cholesterol is esterified by a mucosal esterase (60), which originates in the pancreas. It has been suggested that cholestane-3β,5α,6β-triol suppresses cholesterol absorption by inhibiting the cholesterol esterase (41). In view of the structural similarity between diosgenin and cholesterol (both compounds

[4] It was noted that only 6% of the radioactivity present in neutral lipids was recovered as cholesterol in the intestine (Table 8). We have reported on a similar relationship before (31), and have hypothesized that the rates of one or more of the enzymatic reactions involved in the conversion of neutral sterols to cholesterol are slower in the intestine than in the liver, resulting in the formation of small pools of intermediates (51) sufficient to trap and dilute the radioactivity originating from acetate.

have stereochemically identical rings (A, B, C and D)), we have tested diosgenin in vitro for its possible effect on pancreatic cholesterol esterase; no effect was found and we have concluded that diosgenin does not interfere with cholesterol absorption by inhibiting its esterification.

The effect of diosgenin on cholesterol metabolism in rats can be summarized in the following sequence of events. Diosgenin-induced suppression of absorption of dietary cholesterol and of that undergoing enterohepatic circulation results in increased fecal excretion of neutral sterols. The resultant decrease in the amount of cholesterol that reaches the liver causes a marked increase in the rate of hepatic cholesterol synthesis, primarily by derepression of HMG CoA reductase. This is accompanied by increased secretion of cholesterol into bile, thus contributing to the increase in fecal sterols. Serum cholesterol is unaltered in normal rats because the decrease in the absorption of cholesterol is compensated by an increase in cholesterol synthesis. However, in rats fed cholesterol, diosgenin decreases the elevated levels of cholesterol in serum LDL and increases cholesterol in serum HDL. In contrast to anion-exchange resins, diosgenin has no effect on bile acid excretion or on the levels of bile acids in bile.

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