Effects of synthetic glycosides on steroid balance in *Macaca fascicularis*

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Abstract The predominantly β-anomer of diosgenin glucoside (DG) was synthesized and its effects on cholesterol homeostasis were tested in monkeys. Cynomolgus macaques (*Macaca fascicularis*) were fed, during two 3-week periods, a semipurified diet with 0.1% cholesterol and a similar ration containing 1% DG, respectively. A Chow diet was given for 5 weeks between the experimental periods. Cholesterol and bile acid balance were analyzed during the last week of each semipurified diet. Diosgenin glucoside reduced cholesterolemia from 292 mg/dl to 172 mg/dl, decreased intestinal absorption of exogenous cholesterol from 62.4% to 26.0%, and increased secretion of endogenous cholesterol from −0.8 to 93.5 mg/day. The fecal excretion of neutral steroids rose from 40.7 to 157.3 mg/day; that of bile acids changed, nonsignificantly, from 23.1 to 16.0 mg/day. The cholesterol balance was −44 mg/day in the control period, and 88 mg/day in the DG-fed animals. No toxic signs were observed. Thus, when long-term studies demonstrate that the glucoside is well tolerated, DG and other synthetic glycosides with similar activities may be of use in the management of hypercholesterolemia and atherosclerosis. — **Malinow, M. R., W. H. Elliott, P. McLaughlin, and B. Upson.** Effects of synthetic glycosides on steroid balance in *Macaca fascicularis*. *J. Lipid Res.* 1987. **28:** 1–9.

Supplementary key words cholesterol absorption • fecal sterols • bile acids • saponins • atherosclerosis • diosgenin glucoside

An interaction of saponins and cholesterol was postulated when growth depression in chicks fed alfalfa saponins (1) was overcome by the addition of cholesterol to the diet (2). Further studies showed that alfalfa saponins also reduce cholesterolemia in the chicken (3, 4), whereas alfalfa prevents hypercholesterolemia and modifies atherogenesis in cholesterol-fed rabbits (5, 6); Kritchevsky, Teplor, and Story (7) suggested an involvement of alfalfa saponins in reducing cholesterol absorption. Several investigators have reported the effects of different saponins on cholesterol homeostasis (8–14).

We have shown that alfalfa meal, sun cured alfalfa hay, *Medicago sativa*, reduces hypercholesterolemia and induces regression of atherosclerosis in cholesterol-fed monkeys (15). We demonstrated that an extract of alfalfa meal, operationally termed alfalfa saponins (16), lowers intestinal absorption of cholesterol in rats (17) and monkeys (18). The reduced intestinal cholesterol absorption is not observed with saponin-free alfalfa meal (19). Moreover, alfalfa saponins reduce the hypercholesterolemia expected in monkeys as a result of high-fat, high-cholesterol diets (18, 20, 21), increase the fecal excretion of neutral steroids and probably bile acids (18), prevent atherosclerosis in rabbits (22), and induce the regression of aortic and coronary atherosclerosis in monkeys without any signs of toxicity (21).

Studies with saponins, though, are usually hampered by the complex nature of the plant extracts, making it difficult to isolate a pure saponin in sufficient amounts to conduct experiments in mammals. To study cholesterol homeostasis as affected by pure saponins of known chemical structure, we elected to synthesize glycosides of available sapogenins. We choose tigogenin and its closely related compound diosgenin because the former is the aglycone of certain glycosides found in the saponin digitonin (23); we had previously demonstrated that digitonin

Abbreviations: DG, diosgenin glucoside; TC, tigogenin cellobioside; HPLC, high performance liquid chromatography; SPD, semipurified diet. The following systematic names are given to compounds referred to by trivial names: β-sitosterol, (24R)ethyl-5-cholesten-3β-ol; campesterol, (24R)methyl-5-cholesten-3β-ol; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanic acid; cholesterol, 5-cholesten-3β-ol; cholic acid, 3α,7α,12α-trihydroxy-5β-cholanic acid; coprostanone, 3-oxo-5β-cholestane; deoxycholic acid (I), 3α,12α-dihydroxy-5β-cholanic acid; diosgenin (II), (5α,20α,22α,25R)-spirosten-3β-ol; diosgenin-cellobioside, (II)-4-O-β-D-glucopyranosyl-β-D-glucopyranoside; diosgenin-glucoside, (II)-4-O-β-D-glucopyranoside; 6β-hyodeoxycholic acid, 3α,6β-dihydroxy-5β-cholanic acid; lithocholic acid, 3α-hydroxy-5β-cholanic acid; neotigogenin, (5α,20α,22α,25S)-spirostan-3β-ol; tigogenin (III), (5α,20α,22α,25S)-spirostan-3β-ol; tigogenin cellobioside, (III)-4-O-β-D-glucopyranosyl-β-D-glucopyranoside; tigogenin-glucoside, (III)-4-O-β-D-glucopyranoside; 23-nordeoxycholic acid, 23-nor-(I); ursodeoxycholic acid, 3α,7β-dihydroxy-5β-cholanic acid; yamogenin, (5α,20α,22α,25S)-5-spirosten-3β-ol.

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prevents the expected hypercholesterolemia in cholesterol-fed monkeys (24). Four members of a series of glycosides synthesized in our laboratory, namely diosgenin glucoside (DG), diosgenin cellobioside (DC), tigogenin glucoside (TG), and tigogenin cellobioside (TC), decrease the intestinal absorption of cholesterol in rats more effectively, on a weight basis, than do alfalfa saponins; comparable doses of the aglycones diosgenin and tigogenin are ineffective (25), although diosgenin at higher doses prevents intestinal absorption of cholesterol in rats (26, 27). Of the synthetic compounds, the beta anomer of TC seems to have the greatest activity (Malinow, M. R., J. O. Gardner, J. T. Nelson, P. McLaughlin, B. Upson, and R. Aigner-Held, unpublished observations), but we selected DG for the present studies because it was technically more available. We report here the effects of DG on cholesterol and bile acid balance in cynomolgus macaques.

METHODS

The methods have been described (18) and the previous publication should be consulted for experimental details.

Animals

Six adult female cynomolgus macaques (*Macaca fascicularis*) that had been chow-fed for at least 1 month (Purina Monkey Chow, 15% protein; Ralston Purina Company, St. Louis, MO) were used. The monkeys were housed individually in metabolic cages; food and water were offered ad libitum. The room was kept at 26°C and was lighted from 7 AM to 11 PM. The monkeys were maintained for 3 weeks on a semipurified diet (SPD) (18) plus 1% DG. The diet contained (g/100 g) casein (18.0), sugar (30.0), honey (10.0), coconut oil (8.5), safflower oil (2.5), alphacel (12.0), banana (10.0), and additional vitamins (OWP, ICN Nutritional Biochemicals, Cleveland, OH), salts (Hegsted IV, ICN Nutritional Biochemicals) and cholesterol (0.1%). The percent caloric composition of the diet was protein (20.6), fat (33.5), and carbohydrates (45.9); the cholesterol content was 35 mg/100 Kcal; the food was mixed with agar to facilitate measurement of dietary intake. On day 16, after the morning feeding, an oral dose of labeled cholesterol and β-sitosterol was given to each monkey by nasogastric tube (#8 French; Pharmaseal, Toa Alta, Puerto Rico). The isotopes ($\sim$32 μCi of [1,2-^{3}H]cholesterol and $\sim$12 μCi of [4-^{14}C]β-sitosterol) were dissolved in diethyl ether with 16 mg of cholesterol and 8 mg of β-sitosterol; 8 ml of corn oil was added and mixed, and the ether was evaporated under N_2 overnight. Each monkey was given 1 ml of the corn oil mixture and then 6 ml of water to flush out the nasogastric tube. After the radioactive dose, the daily food intake was measured and the feces were collected for 5 days. Venous blood was taken at the beginning and end of each diet change and also 48 hr after the radioactive dose. Monkeys were weighed at the beginning and end of each dietary period.

A wash-out period of 5 weeks on the chow diet ensued. The above procedure was then repeated, except that the semipurified diet plus 0.1% cholesterol did not contain DG. The experimental design is shown in **Fig. 1.** Results were contrasted by Student's two-tailed paired *t* test.

Chemicals

Cholesterol and β-sitosterol were obtained from Calbiochem-Boehring Corporation, American Hoechst Corporation (San Diego, CA), and Applied Science Labs, Inc. (State College, PA), respectively. The [1,2-$^3H_2$]cholesterol (New England Nuclear, Boston, MA; 46.5 mCi/mmol) and [4-^{14}C]β-sitosterol (Amersham Corporation, Arlington Heights, IL; 58 mCi/mmol) were purified by thin-layer chromatography before use. The DG was synthesized by glucosidation of diosgenin (Syntex Research Inc., Palo Alto, CA) through adaptation of the method of Hanessian and Banoub (28). Since diosgenin was also present as its isomer yamogenin (14% by weight), the synthetic material probably contained a small amount of yamogenin glucoside; however, the substance will be called DG for the sake of brevity. Structures were identified by nuclear magnetic resonance spectographic analysis in H_6-dimethyl sulfoxide (Aldrich Chemical Company, Milwaukee, WI) with a Brucker WM 300 Fourier transform instrument; tetramethylsilane was the internal reference. A 4000 Hz sweep and a 4.0-μsec pulse width (15 μsec = 90° flip angle) were used.

Analytical methods

Plasma cholesterol was determined with a modified version of the $FeCl_3$ method of Rudel and Morris (29). Radioactivity assays were determined in an aliquot of a petroleum ether extract of plasma; plasma had previously been saponified with 33% KOH at 100°C for 1 hr. Assays of radioactivity were carried out by liquid scintillation spectrometry after the addition of suitable scintillation fluid. The figures were computed as disintegrations per minute by an automatic external standardization method (18).

The following serum determinations were made with usual laboratory procedures (AutoAnalyzer, SMA-12; Technicon Corporation, Ardlsey, NY) in weeks 3 and 11, corresponding to the end of each experimental period (Fig. 1): glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, CO_2, uric acid, calcium, phosphorus, total protein, albumin, cholesterol, triglycerides, total bilirubin, direct bilirubin, alkaline phosphatase, lactic dehydrogenase, and serum glutamic oxaloacetic

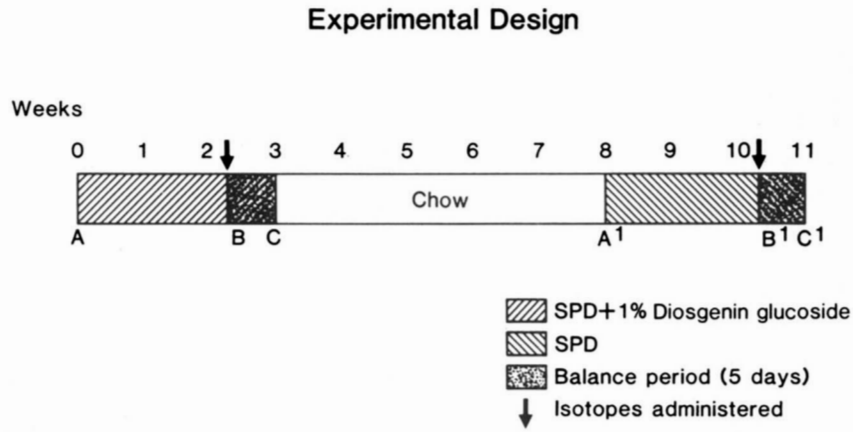

Fig. 1. Experimental design: A, B, C, and A^1, B^1, and C^1 indicate venous bleeding for analyses. SPD, semipurified diet.

transaminase; hematology determinations (erythrocytes and leukocytes) were also made initially and at the end of each dietary period.

The feces, collected daily during the balance period, were pooled for each monkey, weighed, and stored frozen in tared blender jars. For analysis, feces were thawed, an approximately equal volume of water was added, and the mixture was homogenized in an Oster blender. Two aliquots of the homogenate were immediately drawn by pipette into tared 50-ml screw-cap tubes for subsequent analysis. The total fat content of the feces was determined gravimetrically by a modified version (18) of Sobel's method.

Labeled neutral steroids were determined according to reported methods (18). Neutral steroids and bile acids were separated by differential extraction and subsequently purified by thin-layer chromatography. The mass of individual neutral steroids and bile acids was determined by a modification of the gas–liquid chromatographic methods described by Miettinen, Ahrens, and Grundy (30) and Grundy, Ahrens, and Miettinen (31). A Hewlett-Packard model 402 gas chromatograph (GC) fitted with U-shaped silanized glass columns (183 × 0.63 cm, o.d.), a flame ionization detector, and with helium as carrier gas (40 psi at a flow rate of 60 ml/min), and a Hewlett-Packard model 3392A integrator were used. Fecal sterols were separated by thin-layer chromatography (Florisil plates, 20 × 20 cm × 1 mm thick) into three fractions (Miettinen et al. (30)). Fractions I and II were combined and analyzed as free alcohols in hexane with 5α-cholestane (Steraloids, Inc., Wilton, NH) as internal standard on a column packed with 3% OV-210 (Supelco, Inc., Bellefonte, PA) at 210°C. Sterols from Fraction III (30) were derivatized as trimethylsilyl ethers (32) and analyzed on a column packed with SP 2100 (Supelco, Inc.) at 230°C. Bile acids were converted to their methyl ester-trimethyl ethers and analyzed on a column packed with SP2100 at 250°C (32); 23-nor deoxycholic acid (Steraloids, Inc.) was similarly derivatized and used as the internal standard. Bile acid results were expressed as lithocholate, deoxycholate, identified by gas–liquid chromatography, and "other." The identities were investigated by mass spectrometry with an LKB model 9000 GC/MS (LKB Instruments, Inc., Pleasant Hill, CA). The material was derivatized in the same manner as indicated; gas chromatography was performed on a column (183 × 0.63 cm, o.d.) packed with 1% OV-101 (Supelco, Inc.) at 280°C with carrier gas flow at 30 ml/min.

The sterol content of the food (cholesterol, β-sitosterol, and campesterol) was determined by high performance liquid chromatography (HPLC) (33). The HPLC system included a Waters model U6K injector, a model M-45 pump, a model 480 UV detector set at 212 nm, a model 721 programmable system controller, a model 730 data module, and an IBM column (250 × 4.5 mm) packed with C_{18} silica as 5-μm particles. The sterols were dissolved in 50 μl of methylene chloride and an aliquot of 1 μl was injected; the eluting solvent was HPLC-grade methanol.

All analyses were carried out with numbered samples so that the analyst was uninformed as to the nutritional treatment of the animal from which each sample was derived.

Estimation of intestinal absorption of cholesterol and of steroid balance

The radioactive dose received by each monkey was calculated from the weight of the syringe before and after oral infusion; an aliquot of the material was assayed for radioactivity (18). The fecal labeled steroids were assayed in terms of total fecal output of the label by methods described elsewhere (18). Losses caused by degradation of cholesterol to substances not recovered by the method of analysis were estimated from losses of [4-^{14}C]β-sitosterol,

which was used as the standard; absorption of [4-^{14}C]β-sitosterol was minimal (see below), and it was disregarded in the calculations. The excreted labeled neutral steroids were considered to represent the nonabsorbed cholesterol, and thus absorption was expressed as "100-feces," equivalent to the percentage of the infused dose. The steroid balance was based on a simplified model of cholesterol transport (34). We calculated values for nonabsorbed exogenous cholesterol as intake minus intake times the % absorption. The net excretion of endogenous cholesterol was calculated as fecal neutral steroid excretion minus nonabsorbed exogenous cholesterol, i.e., reabsorption was disregarded. Moreover, it was assumed that the absorbed labeled cholesterol was not reexcreted within the time period of the balance procedure and that no measurable isotopic exchange with mucosal cholesterol occurred (see below). The balance was calculated as fecal excretion of neutral steroids plus bile acids minus the cholesterol intake.

RESULTS

Structure of synthetic glycoside (Table 1)

Table 1 shows key features important to the structural assignment of DG based on the proton spectrum of cellobiose reported by Hall, Morris, and Sukumar (35). The data indicate a 85:15 beta:alpha ratio. Acetylated material and other impurities were present in trace amounts; 14% of the sapogenin was in isomeric form as yamogenin. **Fig. 2** shows the proposed structure for the predominant DG (beta anomer). In the structure of yamogenin, the spatial configurations of the CH_3 and H groups at C25 are reversed (not shown).

General results (Tables 2-4)

The monkeys appeared healthy and had similar body weights at the beginning of and throughout each observation period. Food intake was similar with both diets during the balance procedure, although the cholesterol intake was somewhat less during the DG period; the values shown in the table resulted from diet analysis and reflect minor differences between batch preparations. The intakes of plant sterols (around 12 mg/day) were similar for both periods (not shown). Feces weights (not shown) and fecal fat contents were similar in the DG and control periods (**Table 2**). No signs of toxicity were detected; hematologic and plasma chemistry values, except cholesterolemia, were not affected (**Table 3** and **Table 4**).

TABLE 1. Key features of nuclear magnetic resonance spectrum of diosgenin glucoside

Carbon # Assignment	Chemical Shift in (ppm)	No. of Protons
16	4.3 m	1
18	0.74 s	3
19	0.98 s	3
1'	4.81 d; j = 3.5Hz	0.15
1'	4.26 d; J = 7.8Hz	0.85

Abbreviations: m, multiplet; s, singlet; d, doublet; J, coupling constant.

Plasma cholesterol (Table 3)

Plasma cholesterol values with the chow diet were similar at the start of the control and the DG periods: 178 ± 12 and 165 ± 12 mg/dl, respectively (not shown). With the SPD plus 0.1% cholesterol regimen (control period), plasma cholesterol rose to 292 ± 24 mg/dl; however, with the SPD plus 0.1% cholesterol and 1% DG regimen, plasma cholesterol remained at the level of the chow regimen (172 ± 18 mg/dl). Triglyceride levels were not affected.

Steroid balance results (Table 5 and Table 6)

The DG reduced intestinal absorption of cholesterol from 62.4 ± 5.4% to 26.0 ± 3.4% of the infused dose. The amounts of cholesterol radioactivity in plasma 48 hr after the pulse dose were 12.8 ± 2.1 and 2.1 ± 0.2% (P = 0.003) of the infused dose in the control and DG diet periods, respectively (not shown); the amount of β-sitosterol radioactivity was also reduced from 0.28 ± 0.05 to 0.03 ± 0.01% (P = 0.004) of the infused dose (not shown).

Addition of DG to the diet induced the following changes. Endogenous cholesterol net excretion was increased from −0.8 ± 6.5 to 93.5 ± 26.7 mg/day. Neutral steroid fecal excretion rose from 40.7 ± 9.1 to 157.3 ± 28.1 mg/day. Bile acid fecal excretion was changed from 23.1 ± 4.6 to 16.0 ± 2.2 mg/day (not significant). Bile acids listed as "other" (Table 6) were identified as cholate and probably α-muricholate as typical C_{24} acids; the total amount of these acids was estimated as 10% of the materials present in "other." The remaining constituents are represented as C_{27} acids, most of which appear to be metabolites of ingested sterols and diosgenin. Total fecal steroids increased from 63.7 ± 12.3 to 173.3 ± 28.1 mg/day. The steroid balance was negative (−44 ± 10 mg/day) in the control monkeys. In contrast, for monkeys receiving DG, the balance was 88 ± 26 mg/day.

DISCUSSION

Our results extend previous data reported by several authors on the effects of plant glycosides (4, 8-14, 16-18, 20, 24) to substantially pure glycosides of known structure; the present data suggest that the active principles in

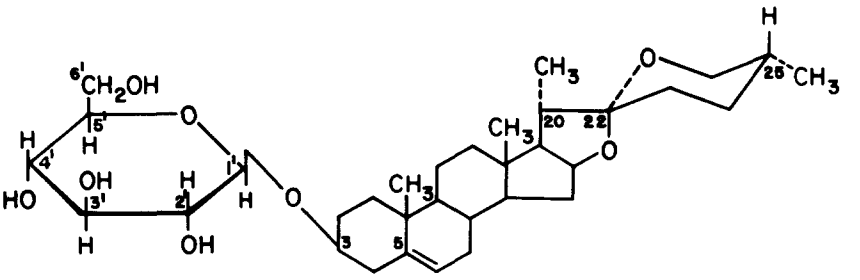

Fig. 2. Structure of diosgenin glucoside (β-anomer).

decreasing cholesterol absorption and cholesterolemia of previous experiments with operationally defined saponins, are indeed saponins.

Grundy (36) and Grundy, Ahrens, and Salen (37) have lucidly discussed limitations of the isotopic methods for determining cholesterol absorption. Our radioactive bolus may not represent food cholesterol absorption throughout a 24-hr period, but comparisons between the two observation periods with identical conditions except for the drug under study should provide reliable estimates. Temporary delay of labeled cholesterol in the intestinal mucosa, which can give misleading results from casual stool specimens, was circumvented by total stool collection over a 5-day span. Finally, the assumption that cholesterol loss through bacterial degradation is compensated for by simultaneous analysis of radioactive β-sitosterol has been validated (37). Moreover, coprostanone or coprostanol derived from [1,2-^{3}H]cholesterol may experience loss of tritium through keto-enol tautomerism (38) and thus may make the apparent absorption values too high, but the magnitude of the difference in the absorption values is so large that the conclusion that DG lowers cholesterol absorption is likely valid.

The steroid balance method for input–output analysis of cholesterol homeostasis (39) requires a steady state. Because of limited DG supplies, we carried out our analysis only 2 weeks into the regimen; most probably at this time cholesterol body pools were increasing, as suggested by the elevated cholesterolemia and the negative balance figures in the control period. These responses would counteract the effects of DG. Moreover, the positive balance during DG ingestion suggests that the glucoside increased total body cholesterol synthesis. Similar findings were obtained from monkeys receiving alfalfa saponins under more steady conditions (18). The results agree with the observation that mice given alfalfa saponins incorporate more [^{14}C]acetate into liver unsaponifiable material (11). Likewise, pigs fed cholestyramine, which presumably interferes with cholesterol absorption through bile acid binding in the intestinal lumen (40), have increased incorporation of [^{14}C]acetate into digitonin-precipitated liver sterols (41). Cholestyramine also accelerates the receptor pathway of cholesterol disposal (42). Whether DG promotes high-affinity low-density lipoprotein receptors and the activity of hydroxymethylglutarate coenzyme A reductase in the liver needs to be established.

For the computation of net excretion of endogenous cholesterol, i.e., cholesterol excreted in the bile and in desquamated intestinal cells, we assumed that reexcretion of the absorbed labeled cholesterol was undetectable within the 5-day fecal collection period. This assumption is in accord with findings in monkeys that no recirculation of

TABLE 2. Balance study in *Macaca fascicularis* (mean ± SEM)

Group	Body Weight		Food Intake	Cholesterol Intake	Fecal Fat
	Initial	Final			
	kg		*g/day (wet wt)*	*mg/day*	*g/day*
Control (n = 6)	3.22 ± 0.18	3.28 ± 0.15	99.69 ± 4.17	108.0 ± 4.5	1.21 ± 0.17
Diosgenin glucoside (n = 6)	3.18 ± 0.16	3.08 ± 0.18	94.20 ± 5.58	85.3 ± 4.7	1.52 ± 0.22
P (paired *t* test)	NS[a]	NS	NS	0.001	NS

[a]NS, Not significant.

TABLE 3. Effects of diosgenin glucoside on hematologic variables and plasma lipids in *Macaca fascicularis*

Variable	Experimental (SPD + DG)	Control (SPD)
Leukocytes ($\times 10^3/mm^3$)	10.22 ± 1.28	9.13 ± 1.05
Erythrocytes ($\times 10^6/mm^3$)	6.40 ± 0.23	6.31 ± 0.22
Hematocrit (%)	38.7 ± 1.3	41.2 ± 1.2
Hemoglobin (g/dl)	11.9 ± 0.4	12.4 ± 0.3
Total cholesterol (mg/dl)	172 ± 18	292 ± 24[a]
Triglycerides (mg/dl)	44 ± 6	41 ± 12

Values given as mean ± SEM; n = 6; SPD, semipurified diet; DG, diosgenin glucoside. See experimental design in Fig. 1 for explanation of intervals.

[a] P (paired t test) = 0.003.

TABLE 4. Serum variables in *Macaca fascicularis*

Variable	Experimental (SPD + DG)	Control (SPD)
Glucose (mg/dl)	66 ± 14	77 ± 10
Blood urea nitrogen (mg/dl)	15.7 ± 1.0	15.2 ± 0.7
Creatinine (mg/dl)	0.8 ± 0.06	0.8 ± 0.05
Sodium (mEq/l)	149 ± 1.0	147 ± 0.7
Potassium (mEq/l)	4.9 ± 0.15	4.9 ± 0.21
Chloride (mEq/l)	109 ± 1	109 ± 1
CO_2 (mEq/l)	17.2 ± 1.2	18.5 ± 0.9
Uric acid (mg/dl)	< 1.0	< 1.0
Calcium (mg/dl)	9.8 ± 0.12	9.9 ± 0.114
Inorganic phosphate (mg/dl)	5.2 ± 0.3	4.9 ± 0.4
Total protein (g/l)	8.0 ± 0.2	8.0 ± 0.2
Albumin (g/l)	4.12 ± 0.07	4.03 ± 0.11
Triglycerides (mg/dl)	44 ± 6	41 ± 12
Total bilirubin (mg/dl)	0.28 ± 0.03	0.20 ± 0.45
Direct bilirubin (mg/dl)	0.05 ± 0.02	0.05 ± 0.22
Alkaline phosphatase (IU/l)	330 ± 81	186 ± 47
Lactic dehydrogenase (IU/l)	266 ± 44	328 ± 67
Serum glutamic oxalacetic transaminase (IU/l)	46 ± 12	62 ± 14

Values given as mean ± SEM; n = 6; SPD, semipurified diet; DG, diosgenin glucoside. Determinations shown were performed at the end of the dietary periods.

orally administered radioactive cholesterol occurs within 10 days of its ingestion (43).

Three phenomena we observed, i.e., that the glucoside prevented the expected rise in plasma cholesterol, decreased exogenous and endogenous cholesterol absorption, and increased fecal excretion of neutral steroids, have also been observed, to lesser degrees, in *M. fascicularis* fed alfalfa saponins (18). Although the monkeys ingested slightly less cholesterol during the DG period than in the control period, it is unlikely that these differences were responsible for the changes, since plasma cholesterol levels did not vary in *M. fascicularis* when the cholesterol content in food was raised by about 50% from an initial concentration equivalent to that used in our studies (44). The present results should be extended to chow-fed animals, as well as to animals fed semipurified diets without added cholesterol; under the last mentioned conditions, synthetic glycosides reduce cholesterolemia in monkeys (Malinow, M. R., P. McLaughlin, and B. Upson, unpublished observations).

The fecal bile acids (Table 6) were expressed as lithocholic (LC) and deoxycholic (DC) acids, products of bacterial degradation of primary bile acids, and "other," to enable a simple comparison with an earlier study (18). Identification of the materials found in "other" suggests that only a minor fraction are typical C_{24} acids. These results are in contrast to our previous report where we concluded that bile acid excretion was increased by alfalfa saponins, based on a large unidentified peak in the gas chromatogram (18). However, the present interpretation is in accord with studies on the disposition of diosgenin which is partly excreted in the bile as acidic metabolites (45). Moreover, the slight increase in fecal fat excretion without clinically apparent steatorrhea, was also observed in monkeys fed alfalfa saponins (18).

The diosgenin used for our synthesis contained yamogenin, the (25S)-isomer of diosgenin, as is usually the case with these plant sapogenins (46). It is unlikely that the (25R) or (25S) glucoside isomers had different activities because a glycoside of neotigogenin, the (25S)-isomer of tigogenin, had similar effects on intestinal absorption of cholesterol in rats, as a tigogenin (25R)-glycoside (Malinow, M. R., P. McLaughlin, and B. Upson, unpublished observations). Since alfalfa saponins are C30 triterpenoid glycosides (16), whereas DG is a C27 spirostane glucoside, the data reported here suggest that saponins with totally different aglycones and sugar moieties alter cholesterol homeostasis. A similar conclusion was reached when digitonin, a mixture of spirostane glycosides (23),

TABLE 5. Balance study in *M. fascicularis* (mean ± SEM)

Group	Cholesterol Intestinal Absorption (% of injected dose)	Fecal Excretion (mg/day)				Calculated Body Cholesterol Balance (mg/day)
		Calculated Endogenous Cholesterol	Neutral Sterols	Bile Acids	Total Sterols	
Control (n = 6)	62.4 ± 5.4	−0.8 ± 6.5	40.7 ± 9.1	23.1 ± 4.6	63.7 ± 12.3	−44 ± 10
Diosgenin glucoside (n = 6)	26.0 ± 3.4	93.5 ± 26.7	157.3 ± 28.1	16.0 ± 2.2	173.3 ± 28.1	88 ± 26
P (paired t test)	0.001	0.003	0.001	NS	0.05	0.01

TABLE 6. Bile acids excreted in feces of *Macaca fascicularis*

Group	Fecal Excretion (mg/day)		
	Lithocholic Acid	Deoxycholic Acid	Other
Control (n = 6)	9.28 ± 2.60	13.46 ± 2.38	0.3 ± 0.1
Diosgenin glucoside (n = 6)	6.00 ± 1.48	6.93 ± 0.78	3.1 ± 0.5
P (paired *t* test)	NS	0.02	0.01

prevented the rise of cholesterolemia expected in cholesterol-fed monkeys (24).

Cayen and Dvornik (26) and Uchida et al. (27) have shown that diosgenin prevents cholesterol absorption in rats. It may be argued that DG is hydrolyzed in the intestine and its effects are due to the free aglycone and not to the glucoside. But at comparable small doses (15 mg/rat), diosgenin does not inhibit cholesterol absorption and DG does (25); the diosgenin doses Cayen and Dvornik (26) and Uchida et al. (27) used were several orders of magnitude larger than the DG doses we used in rats (25). If partial DG hydrolysis does occur, it seems likely that the glucoside is inactivated and small concentrations of the free aglycone do not affect cholesterol homeostasis. The mechanisms through which DG decreases cholesterol absorption have not been elucidated. Previous hypotheses about similar effects of alfalfa saponins (18) include formation of glycoside-cholesterol complexes, interaction between intestinal cells and membrane cholesterol with a consequent decrease in the maximal transport rate or number of transport sites, and an increase in the thickness of the unstirred water layer or its resistance. Additionally, DG may inactivate mucosal enzymes involved in cholesterol absorption, i.e., cholesterol esterase and acyl coenzyme A:cholesterol acyl transferase (47). Whether DG may be absorbed needs to be determined; the intestinal absorption of soybean saponins is minimal (8) as was the case with certain synthetic glycosides, namely, the alpha and beta anomers of [5-^{3}H]tigogenin cellobioside (Malinow, M. R., P. McLaughlin, and B. Upson, unpublished observations).

Of particular importance in our study was the lack of toxic effects of DG as determined by survival, body weight changes, food consumption, general appearance, and multiple blood parameters; similar results were obtained with alfalfa saponins given for protracted periods to rats and monkeys (48, 49). Because alfalfa saponins prevent atherosclerosis (22) and induce regression of established atherosclerosis (21), it is likely that similar effects are brought about by DG and other members of the series of synthetic glycosides that we have investigated. In view of the decrease in coronary heart disease morbidity and mortality following reduction of hypercholesterolemia in patients receiving cholestyramine (50), synthetic glycosides, if devoid of toxicity in long-term studies, may be of use in the clinical management of atherosclerotic disease.

We are thankful to Dr. John A. Edwards, Syntex Research, Inc., Palo Alto, CA, for providing us with a generous amount of diosgenin; to Dr. John O. Gardner and Ms. Janice T. Nelson, Syntex Research Inc., for the analysis of diosgenin and for nuclear resonance spectrum data on DG; and to Mr. Gregory Grochon and Mrs. Ping H. Patrick, St. Louis University School of Medicine, St. Louis, MO, for fecal bile acid and sterol analyses. Publication No. 1499 of the Oregon Regional Primate Research Center, supported by grants RR-00163 and HL-16587 from the National Institutes of Health.

Manuscript received 17 June 1985, in revised form 1 November 1985, and in re-revised form 2 September 1986.

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