Effect of dietary tomatine on cholesterol metabolism in the rat

M. N. CAYEN

Department of Biochemistry, Ayerst Research Laboratories,
Montreal, Quebec, Canada

ABSTRACT Tomatine is a virtually nonabsorbable saponin which has been used as an antifungal agent and analytically as a cholesterol precipitant. It was used in this study to determine whether or not it can form a complex with cholesterol in vivo in the rat intestine and what effects such complex formation would have on cholesterol metabolism. Rats that were fed tomatine as 1% of the diet had a decreased uptake of dietary cholesterol by the liver, an increased rate of hepatic and intestinal cholesterol synthesis as well as a partial offsetting of the dietary cholesterol-induced decrease in hepatic cholesterogenesis, and an apparent increase in sterol excretion without an effect on bile acid excretion. In vitro, tomatine did not sequester cholic acid as did cholestyramine. The results show that tomatine has an effect on cholesterol absorption and on other aspects of lipid metabolism in the rat similar to that of cholestyramine, with the notable exception that tomatine increased sterol excretion while cholestyramine increased bile acid excretion. It was suggested that tomatine forms a nonabsorbable complex with cholesterol in the rat intestine.

SUPPLEMENTARY KEY WORDS   saponin · cholesterol absorption · cholesterol biosynthesis · sterol excretion · bile acid excretion · liver · intestine · serum · phospholipids · cholestyramine

A PROMISING APPROACH to the lowering of serum cholesterol levels in patients with type II hyperlipoproteinemia is the development of agents which would interfere with the absorption of cholesterol from the gastrointestinal tract. For example, the resin cholestyramine sequesters bile acids in the gut (1), and thus the increased rate of bile acid excretion results in increased cholesterol catabolism and a lowering of serum cholesterol levels in

man (2). The plant sterol β-sitosterol apparently exerts its cholesterol-lowering action by competing with cholesterol for the cholesterol absorption sites (3, 4).

Another potentially useful approach may be the development of an agent which would form a nonabsorbable complex with cholesterol itself without affecting bile acids, which may be required for the absorption of other dietary constituents. One possible agent is tomatine, a glycoside of a steroidal alkaloid, which, like digitonin, has been used analytically to precipitate cholesterol (5, 6). Tomatine has been used orally as an antifungal agent (7) and, on intramuscular injection, has been reported to have antiinflammatory activity (8). However, on oral administration, there is little evidence of absorption or toxicity (9).

In the present study, the effect of tomatine (I) on various aspects of cholesterol metabolism was studied in order to determine whether the saponin forms a complex with cholesterol when it is fed to the rat. Its action was also compared with that of cholestyramine.

MATERIALS AND METHODS

Animals

Male albino rats (Charles River), weighing 120–140 g, were kept under observation for 7 days prior to each experiment. Only animals with normal food intake and weight gain were used. Dietary regimens comprised Purina Laboratory Chow containing supplements as indicated.

Bile Acid Precipitation in Vitro

The method was similar to that used previously (10). Sodium cholate (100 mg) was added to 20 ml of 0.1 M potassium phosphate buffer (pH 6.2) containing approximately 0.002 μCi of cholic acid-24-^{14}C per ml of buffer.

I
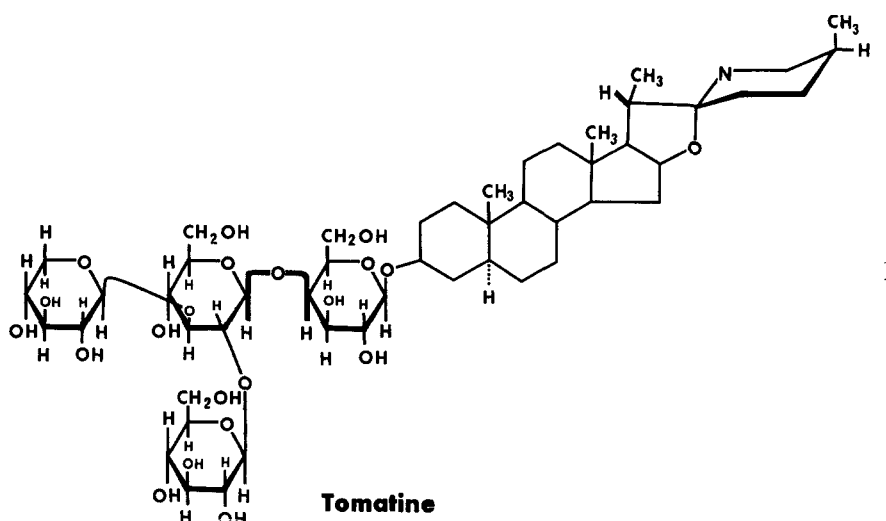
Tomatine

The tests were performed in 40-ml centrifuge tubes. Cholestyramine or tomatine (100 mg) was then added, the mixture was shaken on a mechanical shaker for 1 hr, centrifuged, and the radioactivity content in an aliquot of the supernatant was measured. Preliminary studies showed that the bile acid content of the supernatant which was determined spectrophotometrically (11) was identical to that obtained by measuring the radioactivity. Thus the radioactivity content of the supernatant represents the amount of cholic acid which was not bound by the precipitating agent.

Measurement of Hepatic and Intestinal Cholesterogenesis

Rats were decapitated and livers and intestines were excised and immersed in ice-cold isotonic saline. Intestines (the distal 20 cm) were flushed thoroughly with ice-cold Krebs-Ringer bicarbonate buffer (pH 7.4). Sections were incubated for 1 hr at 37°C in buffer containing acetate-2-14C alone or simultaneously with mevalonate-3H (12). 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 (nonsaponifiable lipids) were extracted with *n*-hexane. Cholesterol was then isolated as its 5,6-dibromo derivative as described previously (13). Liver homogenates were incubated for 1 hr at 37°C in potassium phosphate buffer (pH 7.4) containing labeled precursors and appropriate cofactors, and the cholesterogenic activity was measured in a similar manner (14).

Measurement of Lipid Levels

Total cholesterol levels were measured in the following manner. Serum (0.5 ml) was treated for 1 hr at 45°C with 5 ml of alcoholic KOH while tissue samples were hydrolyzed for 30 min at 70–80°C in alcoholic KOH.

After addition of 4.5 ml of water, the mixture was extracted with 10 ml of hexane. An aliquot of the hexane extract was evaporated to dryness and the residue was taken up in 2 ml of isopropanol. Cholesterol levels were then determined according to Zlatkis, Zak, and Boyle (15) as modified for the AutoAnalyzer (method Np-24).

Phospholipids were determined by the method of Kraml (16), and triglycerides were measured by the semiautomated method of Kraml and Cosyns (17). Total nitrogen in liver homogenates was determined by the Kjeldahl digestion procedure as adapted for the AutoAnalyzer (18). Serum lipoproteins were separated with dextran sulfate (19).

Fecal Sterol and Bile Acid Excretion

Fecal sterol and bile acid fractions were prepared by a modification of the method of Bongiovanni (20). Daily samples were lyophilized, weighed, ground to a powder, and extracted for 24 hr at 75–80°C with 95% ethanol. The ethanol extract was evaporated to dryness, and the residue was dissolved in 10 ml of 15% KOH and hydrolyzed in an autoclave at 120°C for 4 hr. The mixture was then exhaustively extracted with ether to remove the sterol fraction. The remaining hydrolysate was acidified and extracted with ether to remove the bile acids. Recovery studies established a virtually complete separation of cholesterol and cholic acid.

Sources of Compounds

Sodium acetate-2-14C, acetate-3H, DL-mevalolactone-3H, and cholesterol-U-3H were purchased from Amersham/Searle Corp., Arlington Heights, Ill.; cholic acid-24-14C was obtained from New England Nuclear Corp., Boston, Mass. The mevalolactone was treated with dilute methanolic KOH and neutralized to obtain free mevalonic acid. Cholesterol, used as carrier and for feeding, was purified via its 5,6-dibromo derivative (21). Tomatine

was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Cholestyramine (Cuemid) was kindly supplied by Dr. J. Mailloux of Merck Sharp & Dohme of Canada, Montreal.

Radioactivity Measurement

A Packard Tri-Carb liquid scintillation counter, model 3375, was used. Counting efficiency for ^{14}C and ^{3}H was approximately 80% and 36–38% in singly labeled experiments, and 50–60% and 20–25% in doubly labeled studies, respectively.

EXPERIMENTAL

Effect of Tomatine on Uptake of Cholesterol by Liver in Cholesterol-fed Rats

If tomatine forms an insoluble complex with cholesterol in vivo as it does in vitro, then tomatine administered orally to cholesterol-fed rats should prevent cholesterol absorption. This preliminary study was performed to determine whether dietary tomatine affects the uptake of dietary cholesterol by the liver. Rats were fed Purina Chow supplemented with 1% cholesterol, containing 0.1 μCi of cholesterol-^{3}H/g of chow, for 7 days. Diets were also supplemented with tomatine or cholestyramine at levels of 0.5 or 2.0%. Animals were decapitated, and the livers were excised, homogenized, and thoroughly extracted with ethanol–ether 3:1. The radioactivity content of this extract would be due to cholesterol-^{3}H and its metabolites and would depend upon the amount of dietary cholesterol taken up by the liver as well as any changes in cholesterol turnover.

The results are presented in Table 1. Rats fed 2% tomatine did not show any signs of toxicity; body weight gain and liver weight were normal. Equivalent single daily doses were found by Wilson, Poley, and DeEds (9) to be toxic in female weanling rats. Possibly, the lack of toxicity in the present study was due to differences in age

or sex or to the fact that tomatine was administered over a 24-hr period rather than as single daily doses. At a level of 2.0% of the diet, tomatine markedly reduced both the radioactivity and cholesterol content of the liver. Liver cholesterol was only slightly reduced in rats fed 0.5% tomatine. Changes in the radioactivity content paralleled those of cholesterol, since dpm/mg of liver cholesterol remained unchanged. However, in rats fed a diet containing 2% cholestyramine, the radioactivity content was decreased to a greater degree than cholesterol; no explanation is offered for this unusual observation.

This exploratory study thus showed that dietary tomatine altered cholesterol absorption and/or turnover to at least the same extent as did cholestyramine.

Effect of Tomatine on Bile Acid Precipitation in Vitro

In view of the similarity of the effects of tomatine and cholestyramine, a study was carried out to determine whether tomatine can precipitate bile acids in vitro. The results in Table 2 show that, under the experimental conditions used, cholestyramine bound 55% of the cholic acid, while no cholic acid was bound by tomatine.

Effect of Tomatine on Cholesterol Biosynthesis and Lipid Levels

The bile acid sequestering action of cholestyramine increases bile acid excretion (22) and decreases cholesterol absorption, thus causing an increase in both hepatic (22, 23) and intestinal (24) cholesterol synthesis. The rat readily compensates for the increased fecal excretion of bile acids by a corresponding increase in cholesterol biosynthesis; hence, cholestyramine does not lower serum cholesterol levels in the rat (22). Since man cannot increase sterol synthesis sufficiently to compensate for the increased fecal excretion of bile acids, the resin is hypocholesterolemic in man (25). Thus, the rat can be used as an experimental model to demonstrate a cholestyramine-like mechanism of action by measuring the rate of hepatic cholesterol synthesis, even though the agent may

TABLE 1   UPTAKE OF DIETARY CHOLESTEROL-^{3}H BY LIVER IN TOMATINE- AND CHOLESTYRAMINE-FED RATS

Group	Food Intake	Liver Radioactivity		Liver Cholesterol
	g/group/day	dpm/g liver	dpm/mg sterols	mg/100 g
Control	126 ± 5.2	110,900 ± 9,410	15,400 ± 772	719 ± 50
0.5% Tomatine	113 ± 7.8	80,300 ± 8,970	15,800 ± 1250	526 ± 47*
2.0% Tomatine	115 ± 8.4	44,600 ± 5,820†	14,000 ± 1740	318 ± 20†
0.5% Cholestyramine	116 ± 9.9	99,200 ± 14,760	15,700 ± 2010	654 ± 91
2.0% Cholestyramine	100 ± 8.3*	43,700 ± 7,550†	10,800 ± 1180‡	393 ± 31†

All diets were supplemented with 1% cholesterol containing 0.1 μCi of cholesterol-^{3}H/g of chow. Animals were treated for 7 days. Results are presented as mean ± standard error for six rats per group.
* $P < 0.05$.
† $P < 0.001$.
‡ $P < 0.01$.

TABLE 2   Effect of Tomatine and Cholestyramine on Bile Acid Precipitation in Vitro

Agent	dpm/Total Supernatant	% Bound
Control	99,340	—
Tomatine	98,870	1%
Cholestyramine	44,980	55%

Supernatant radioactivity represents cholic acid which was not bound by precipitating agent. Results presented are averages of triplicate determinations.

not affect circulating cholesterol levels in this species.

It was of interest, therefore, to determine what effect tomatine would have on hepatic and intestinal cholesterogenesis as well as on lipid levels in the rat. Rats were fed for 14 days Purina Chow supplemented with 1% tomatine. Liver homogenates and intestinal sections were incubated simultaneously with acetate-2-^{14}C and mevalonate-^{3}H, and the incorporation into neutral lipids (i.e., hexane-soluble, nonsaponifiable lipids) and cholesterol was determined (13, 14). Serum and liver lipid levels were also measured.

The effect of 1% tomatine on hepatic and intestinal cholesterol synthesis is presented in Tables 3 and 4,

respectively. Tomatine increased the rate of acetate incorporation into neutral lipids and cholesterol in both tissues; the amount of increase was more pronounced in the liver. Tomatine did not affect mevalonate incorporation into neutral lipids in the liver, but did increase mevalonate incorporation into cholesterol. This, coupled with the observation that the increase in hepatic cholesterol synthesis from acetate was almost three times greater than the increase in neutral lipid formation from acetate, shows that, in the liver, tomatine increases the rate of cholesterogenesis primarily at a site between acetate and mevalonate, and at a secondary site after neutral lipid formation. The results obtained with mevalonate and intestinal sections were included in Table 4, but they may not be meaningful because of the limited uptake of mevalonate at sites of intestinal cholesterol synthesis (12, 26).

The effect of dietary tomatine on lipid levels is presented in Tables 5 and 6. Tomatine slightly decreased cholesterol levels in both serum lipoprotein fractions; liver and intestine cholesterol levels were unchanged. Slight decreases of phospholipid levels in low density lipoproteins and of serum triglycerides were also observed.

TABLE 3   Effect of Dietary Tomatine on Hepatic Cholesterol Synthesis

Group	Weight Gain	Neutral Lipids*		Cholesterol	
		Acetate-^{14}C	Mevalonate-^{3}H	Acetate-^{14}C	Mevalonate-^{3}H
	g/rat/14 days	dpm/mg nitrogen			
Control	99 ± 4.3	4,760 ± 875	22,800 ± 740	1,250 ± 324	9,180 ± 954
Tomatine	84 ± 10.8	30,800 ± 3050†	21,600 ± 540	21,530 ± 1755†	16,990 ± 728†
Difference		+530%		+1620%	+85%

Rats were fed for 14 days Purina Chow supplemented with 1% tomatine. Animals were decapitated and liver homogenates were prepared and incubated simultaneously with 1.35 μCi (0.03 μmole) of acetate-2-^{14}C and 0.09 μCi (0.002 μmole) of mevalonate-^{3}H and appropriate cofactors as described previously (14). Cholesterol was isolated, purified, and counted as its dibromo derivative. Results are presented as mean ± standard error for nine rats per group.
* Hexane-soluble, nonsaponifiable lipids.
† P < 0.001.

TABLE 4   Effect of Dietary Tomatine on Intestinal Cholesterol Synthesis

Group	Neutral Lipids		Cholesterol	
	Acetate-^{14}C	Mevalonate-^{3}H	Acetate-^{14}C	Mevalonate-^{3}H
	dpm/g tissue			
Control	35,200 ± 4090	(2190 ± 220)*	2610 ± 443	(225 ± 65)*
Tomatine	106,700 ± 8190†	(5930 ± 738)†	7510 ± 783†	(457 ± 80)‡
Difference	+200%	(+165%)	+190%	(+100%)

Rats were the same as those described for Table 3. Intestinal sections were prepared and incubated simultaneously with 2.8 μCi (0.06 μmole) of acetate-2-^{14}C and 0.3 μCi (0.01 μmole) of mevalonate-^{3}H as described previously (13). Cholesterol was isolated, purified, and counted as its dibromo derivative. Results are presented as mean ± standard error for nine rats per group.
* Results are placed in parentheses due to limited uptake of mevalonate at the site of intestinal cholesterol synthesis (13, 26).
† P < 0.001.
‡ P < 0.05.

TABLE 5   EFFECT OF DIETARY TOMATINE ON CHOLESTEROL LEVELS

		Cholesterol Levels			
		Serum			
Group	LDL*	HDL*	Total	Liver	Intestine
		mg/100 ml			mg/100 g
Control	24.1 ± 1.2	29.8 ± 1.1	53.8 ± 1.9	218 ± 4.1	115 ± 11.3
Tomatine	19.9 ± 0.9†	24.4 ± 1.3‡	45.3 ± 1.4‡	211 ± 4.6	135 ± 14.3

Rats were fed for 14 days Purina Chow supplemented with 1% tomatine. Results are presented as mean ± standard error for 15 rats per group.
 * LDL, low density lipoprotein; HDL, high density lipoprotein. Serum lipoproteins were separated with dextran sulfate (19).
 † $P < 0.05$.
 ‡ $P < 0.01$.

TABLE 6   EFFECT OF DIETARY TOMATINE ON PHOSPHOLIPID AND TRIGLYCERIDE LEVELS

		Phospholipid Phosphorus			Triglyceride Glycerol	
		Serum				
Group	LDL*	HDL*	Total	Liver	Serum	Liver
		mg/100 ml		mg/100 g	mg/100 ml	mg/100 g
Control	1.69 ± 0.07	3.44 ± 0.11	5.13 ± 0.16	106 ± 1.6	14.2 ± 1.4	59.0 ± 3.4
Tomatine	1.45 ± 0.06†	3.25 ± 0.10	4.69 ± 0.12†	111 ± 2.9	9.3 ± 1.2†	50.4 ± 2.7

Rats were the same as those described for Table 5. Results are presented as mean ± standard error for 15 rats per group.
 * LDL, low density lipoprotein; HDL, high density lipoprotein. Serum lipoproteins were separated with dextran sulfate (19).
 † $P < 0.05$.

Effect of Tomatine on Dietary Cholesterol-induced Decrease in Hepatic Cholesterol Synthesis

Since dietary cholesterol suppresses hepatic cholesterol synthesis (27) and since cholesterol intake regulates the rate of cholesterogenesis in rat liver (28), the suggested cholesterol-complexing action of tomatine should partially offset the cholesterol-induced decrease in hepatic cholesterol synthesis. In this study, diets were supplemented for 14 days with 1% cholesterol, 1% tomatine, or 1% cholesterol and 1% tomatine. Animals were decapitated and the incorporation of acetate-2-^{14}C and mevalonate-^{3}H into neutral lipids and cholesterol by liver homogenates was determined as described previously.

The results in Table 7 show that cholesterol decreased while tomatine increased hepatic cholesterol synthesis. The site of tomatine action was again primarily between acetate and mevalonate, with a secondary site observed after neutral lipid formation. Dietary cholesterol inhibited cholesterol synthesis also at two sites. The incorporation of the two precursors into neutral lipids and cholesterol in liver homogenates of rats fed both cholesterol and tomatine was higher than that of rats fed cholesterol alone. Thus, tomatine did indeed partially offset the cholesterol-induced decrease in hepatic cholesterol synthesis.

Effect of Dietary Tomatine on Cholesterol and Bile Acid Excretion

In order to obtain more information as to whether tomatine forms a complex with cholesterol in the gut, studies were carried out to determine the effect of dietary tomatine on fecal excretion of sterols and bile acids. Rats were housed in individual metabolism cages and fed a cholesterol-free diet (29) for the rest of the study. The diet (containing less than 50 μg of sterols/g) comprised 70% infant cereal (Pablum, Mead Johnson & Co., Toronto, Ontario, Canada), 7% wheat germ, 21% skim milk powder, and 2% vitamin mix. The diet was readily accepted by the rats. Animals were kept under observation for 1 wk, after which tomatine was added to the experimental group as 1% of the diet (at the expense of the cereal); control rats continued to receive the cholesterol-free diet. 7 days later, both control and tomatine-treated animals received a single intraperitoneal injection of 4.4 μCi of cholic acid-24-^{14}C and 2.8 μCi of cholesterol-U-^{3}H, and dietary regimens were continued for an additional 7 days. Daily fecal collections were frozen pending analysis. Sterol and bile acid fractions were prepared and the radioactivity contents were measured.

The results presented in Figs. 1–3 are expressed as mean cumulative fecal radioactivity content of the sterol or bile acid fraction for each of the 7 days following in-

TABLE 7   EFFECT OF TOMATINE ON CHOLESTEROL-INDUCED DECREASE IN HEPATIC CHOLESTEROL SYNTHESIS

Group	Weight Gain	Liver Weight	Neutral Lipids		Cholesterol	
			Acetate-¹⁴C	Mevalonate-³H	Acetate-¹⁴C	Mevalonate-³H
	g/rat/14 days	*g*	*dpm/mg nitrogen*			
Control	76 ± 3.5	11.3 ± 0.25	6,100 ± 1,120	27,370 ± 1,270	1,570 ± 359	10,250 ± 712
Cholesterol	72 ± 2.6	11.1 ± 0.31	390 ± 71*	4,040 ± 732*	25 ± 6*	116 ± 36*
Tomatine	78 ± 4.5	11.1 ± 0.42	20,810 ± 1,210*	22,980 ± 665†	14,170 ± 1,790*	17,930 ± 1,210*
Cholesterol + Tomatine‡	73 ± 4.7	11.9 ± 0.33	1,790 ± 389†	18,150 ± 1,660*	202 ± 62§	1,280 ± 217*

Rats were fed Purina Chow supplemented as indicated for 14 days. Liver homogenates were incubated simultaneously with 4.7 μCi (0.10 μmole) of acetate-2-¹⁴C and 0.20 μCi (0.05 μmole) of mevalonate-³H (14). Cholesterol was isolated, purified, and counted as its dibromo derivative. Results are presented as mean ± standard error for eight rats per group.
* $P < 0.001$.
† $P < 0.01$.
‡ Significance levels compared with rats receiving 1% cholesterol.
§ $P < 0.05$.

jection of the radioactive tracers. There were four rats per group. Standard errors are given in Fig. 1 only; in Figs. 2 and 3 there was no difference between any of the treated and control values, and thus no standard errors are presented. The results in Fig. 1 show that there was a dramatic increase in fecal excretion of ³H in the sterol fraction in the tomatine-treated rats (no ¹⁴C was recovered in this fraction). Fig. 2 shows results obtained with fecal bile acid-³H (which was presumed to be formed in the rat from the injected cholesterol-³H), and fecal excretion of bile acid-¹⁴C is presented in Fig. 3. In contrast to the results obtained for fecal ³H in the sterol fraction, tomatine had no effect on the excretion of ¹⁴C- or ³H-labeled bile acid.

DISCUSSION AND CONCLUSIONS

In all the experiments described in this study, tomatine was well tolerated by the rats. Food intake, liver weight,

and body weight gain were normal. The results suggest that dietary tomatine forms a nonabsorbable complex with cholesterol in the gut and has no effect on bile acids. In vitro, cholestyramine precipitated bile acids but tomatine had no effect. The fecal excretion data suggest that tomatine increased sterol excretion but did not alter bile acid excretion. This conclusion is based on the assumption that the injected isotopes became part of the body pools of cholesterol and cholic acid (30). Since the rats in this experiment were fed a cholesterol-free diet, the increased fecal sterols cannot be of dietary origin but must be of endogenous origin. Thus, the principal difference between the action of cholestyramine and tomatine is that cholestyramine increases bile acid excretion (30) while tomatine increases sterol excretion.

These experiments also show that certain effects of tomatine and cholestyramine in the rat are similar. First, both decrease cholesterol absorption, although probably by different mechanisms: cholestyramine sequesters bile
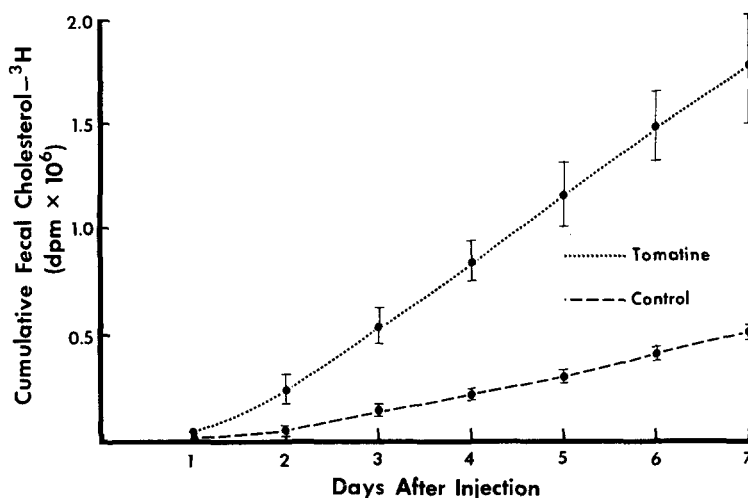

FIG. 1. Fecal cholesterol-³H excretion in tomatine-treated rats injected with cholesterol-³H and cholic acid-24-¹⁴C.
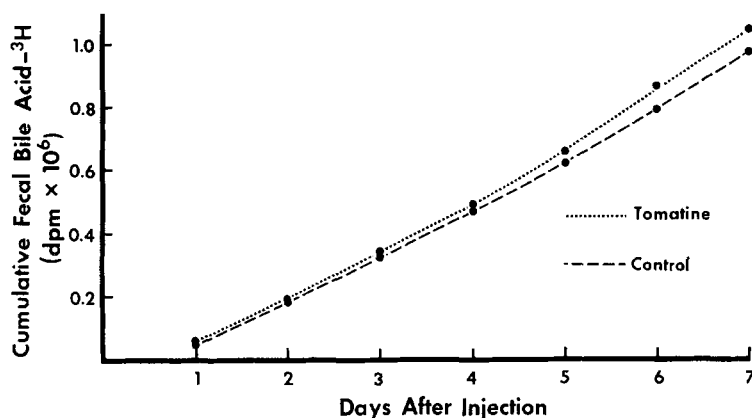
Fig. 2. Fecal bile acid-³H excretion in tomatine-treated rats injected with cholesterol-³H and cholic acid-24-¹⁴C.
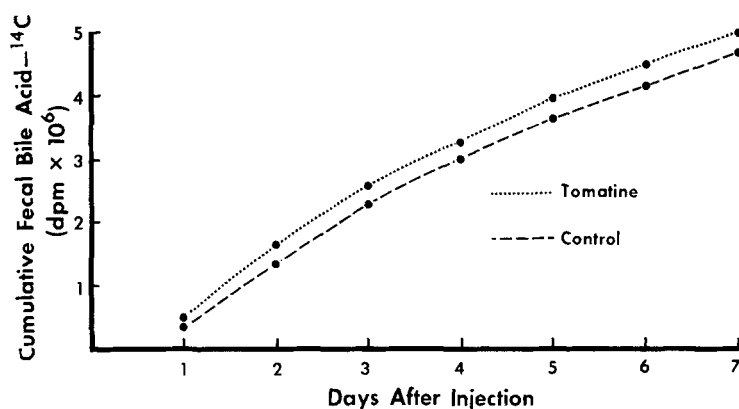

Fig. 3. Fecal bile acid-¹⁴C excretion in tomatine-treated rats injected with cholesterol-³H and cholic acid-24-¹⁴C.

acids which are required for cholesterol absorption, while tomatine probably complexes cholesterol. Second, both agents increase hepatic and intestinal cholesterol synthesis and, like cholestyramine (26), tomatine also partially offsets the dietary cholesterol-induced decrease in hepatic cholesterogenesis. In this study, tomatine reduced only slightly the serum levels of cholesterol, phospholipids, and triglycerides. It must be emphasized that, although statistically significant, these changes were small. Additional experiments (not reported here) have shown that tomatine does not consistently lower serum lipids. It has been demonstrated with cholestyramine (26) that the rat is not a suitable experimental animal to demonstrate that agents which suppress cholesterol absorption cause a consistent decrease in serum lipid levels. The rat compensates for the decreased rate of sterol absorption by a corresponding increase in cholesterol biosynthesis and thus serum lipids may not be affected.

The observation that tomatine increased both hepatic and intestinal cholesterol synthesis is of considerable interest. Both bile acids and cholesterol undergo enterohepatic circulation. The cholestyramine-fed rat compensates for the increased fecal excretion of bile acids by a corresponding increase in bile acid formation from cholesterol as well as an increase in cholesterol biosynthesis; thus the rate of bile acid turnover is enhanced. Tomatine, however, has no apparent effect on bile acid turnover. If tomatine increases sterol excretion by forming a complex with cholesterol in the gastrointestinal tract, then the increase in hepatic cholesterol synthesis in tomatine-treated rats can be regarded as being due to a reduction of the amount of cholesterol reaching the liver via the enterohepatic circulation. This conclusion is substantiated by the observation that dietary cholesterol offsets the tomatine-induced increase in hepatic cholesterol synthesis (Table 7), and supports the evidence of Weis and Dietschy (31) that the enterohepatic circulation of cholesterol regulates the rate at which cholesterol is synthesized by the liver.

The increase in intestinal cholesterol synthesis in tomatine-fed rats also deserves comment. The controlling mechanisms of intestinal cholesterol synthesis appear to be different from those of the liver. It is believed that circulating bile acids control cholesterol synthesis in the

intestine (28) while circulating cholesterol regulates hepatic cholesterol synthesis (28, 31). Thus, since dietary cholesterol has little (28) or no (32, 33) inhibitory effect on intestinal cholesterogenesis, it has been suggested that the "negative feedback" control is absent in the intestine. On the other hand, certain aspects of hepatic and intestinal cholesterogenesis are similar. For example, agents which inhibit cholesterol synthesis in the liver in vitro also do so in the intestine (13). Also, dietary cholesterol is capable of suppressing intestinal cholesterol formation in cholestyramine-treated rats (26); this effect was ascribed to the bile acid sequestering action of cholestyramine and the replacement of bound bile acids by those synthesized from cholesterol of dietary origin. Since tomatine has no apparent effect on bile acid turnover, the tomatine-induced increase in intestinal cholesterol synthesis was an unexpected finding. Possibly, the rate of cholesterol turnover has a greater role in the regulation of intestinal cholesterogenesis than has been previously considered. The definition of this possible role awaits further study.

It is suggested that tomatine interferes with cholesterol absorption and increases sterol excretion by forming a nonabsorbable complex with cholesterol in the gastrointestinal tract.

REFERENCES

1. Tennent, D. M., H. Siegel, M. E. Zanetti, G. W. Kuron, W. H. Ott, and F. J. Wolf. 1960. Plasma cholesterol lowering action of bile acid binding polymers in experimental animals. *J. Lipid Res.* **1:** 469–473.
2. Datta, D. V., and S. Sherlock. 1963. Treatment of pruritus of obstructive jaundice with cholestyramine. *Brit. Med. J.* **1:** 216–219.
3. Best, M. M., C. H. Duncan, E. J. Van Loon, and J. D. Wathen. 1955. The effects of sitosterol on serum lipids. *Amer. J. Med.* **19:** 61–70.
4. Best, M. M., and C. H. Duncan. 1956. Modification of abnormal serum lipid patterns in atherosclerosis by administration of sitosterol. *Ann. Intern. Med.* **45:** 614–622.
5. Kabara, J. J., J. T. McLaughlin, and C. A. Riegel. 1961. Quantitative microdetermination of cholesterol using tomatine as precipitating agent. *Anal. Chem.* **33:** 305–307.
6. Eskelson, C. D., A. L. Dunn, and C. R. Cazee. 1967. Effects of tomatine on the colorimetric determination of cholesterol by the Zak procedure. *Clin. Chem.* **13:** 468–474.
7. Fontaine, T. D., G. W. Irving, Jr., R. Ma, J. B. Poole, and S. P. Doolittle. 1948. Isolation and partial characterization of crystalline tomatine, an antibiotic agent from the tomato plant. *Arch. Biochem.* **18:** 467–475.
8. Filderman, R. B., and B. A. Kovacs. 1969. Anti-inflammatory activity of the steroid alkaloid glycoside, tomatine. *Brit. J. Pharmacol.* **37:** 748–755.
9. Wilson, R. H., G. W. Poley, and F. DeEds. 1961. Some pharmacologic and toxicologic properties of tomatine and its derivatives. *Toxicol. Appl. Pharmacol.* **3:** 39–48.
10. Cayen, M. N. 1970. Agents affecting lipid metabolism. XXXVIII. Effect of neomycin on cholesterol biosynthesis and bile acid precipitation. *Amer. J. Clin. Nutr.* **23:** 1234–1240.
11. Mosbach, E. H., H. J. Kalinsky, E. Halpern, and F. E. Kendall. 1954. Determination of deoxycholic acid and cholic acid in bile. *Arch. Biochem. Biophys.* **51:** 402–410.
12. Dvornik, D. 1968. Agents affecting lipid metabolism. XXXVI. Effect of N-cyclohexyl linoleamide on hepatic cholesterogenesis in vitro. *Can. J. Biochem.* **46:** 1430–1432.
13. Cayen, M. N., and D. Dvornik. 1968. Inhibition of intestinal cholesterogenesis in vitro. *Proc. Soc. Exp. Biol. Med.* **127:** 117–120.
14. Cayen, M. N., and D. Dvornik. 1968. Agents affecting lipid metabolism. XXXI. Properties of an inhibitor of cholesterol synthesis present in rabbit liver mitochondria. *Can. J. Biochem.* **46:** 179–187.
15. Zlatkis, A., B. Zak, and A. J. Boyle. 1953. A new method for the direct determination of serum cholesterol. *J. Lab. Clin. Med.* **41:** 486–492.
16. Kraml, M. 1966. A semi-automated determination of phospholipids. *Clin. Chim. Acta.* **13:** 442–448.
17. Kraml, M., and L. Cosyns. 1969. A semi-automated determination of serum triglycerides. *Clin. Biochem.* **2:** 373–380.
18. Ferrari, A. 1960. Nitrogen determination by a continuous digestion and analysis system. *Ann. N.Y. Acad. Sci.* **87:** 792–800.
19. Hill, P., and D. Dvornik. 1969. Agents affecting lipid metabolism. XXXVII. Separation of rat serum lipoproteins with dextran sulfate. *Can. J. Biochem.* **47:** 1043–1047.
20. Bongiovanni, A. M. 1965. Bile acid content of gallbladder of infants, children, and adults. *J. Clin. Endocrinol.* **25:** 678–685.
21. Schwenk, E., and N. T. Werthessen. 1952. Studies on the biosynthesis of cholesterol. III. Purification of C^{14}-cholesterol from perfusions of livers and other organs. *Arch. Biochem. Biophys.* **40:** 334–341.
22. Huff, J. W., J. L. Gilfillan, and V. M. Hunt. 1963. Effect of cholestyramine, a bile acid binding polymer on plasma cholesterol and fecal bile acid excretion in the rat. *Proc. Soc. Exp. Biol. Med.* **114:** 352–355.
23. Schneider, D. L., D. G. Gallo, and H. P. Sarett. 1966. Effect of cholestyramine on cholesterol metabolism in young adult swine. *Proc. Soc. Exp. Biol. Med.* **121:** 1244–1248.
24. Dietschy, J. M. 1967. The role of bile salts in controlling the rate of intestinal cholesterogenesis. *J. Clin. Invest.* **46:** 1050. (Abstr.)
25. Bergen, S. S., Jr., T. B. Van Itallie, D. M. Tennent, and W. H. Sebrell. 1959. Effect of an anion exchange resin on

serum cholesterol in man. *Proc. Soc. Exp. Biol. Med.* **102:** 676–679.

26. Cayen, M. N. 1969. The effect of starvation and cholesterol feeding on intestinal cholesterol synthesis in the rat. *Biochim. Biophys. Acta.* **187:** 546–554.
27. Gould, R. G. 1951. Lipid metabolism and atherosclerosis. *Amer. J. Med.* **11:** 209–227.
28. Dietschy, J. M. 1968. The role of bile salts in controlling the rate of intestinal cholesterogenesis. *J. Clin. Invest.* **47:** 286–300.
29. Kritchevsky, D., and S. A. Tepper. 1966. Effect of benzyl *N*-benzyl carbethoxyhydroxamate on cholesterol metabolism in the rat. *Proc. Soc. Exp. Biol. Med.* **121:** 1162–1165.
30. Beher, W. T., B. Rao, M. E. Beher, and J. Bertasius. 1967. Bile acid synthesis in normal and hypophysectomized rats: a rate study using cholestyramine. *Proc. Soc. Exp. Biol. Med.* **124:** 1193–1197.
31. Weis, H. J., and J. M. Dietschy. 1969. Failure of bile acids to control hepatic cholesterogenesis: evidence for endogenous cholesterol feedback. *J. Clin. Invest.* **48:** 2398–2408.
32. Gould, R. G., C. B. Taylor, J. S. Hagerman, I. Warner, and D. J. Campbell. 1953. Cholesterol metabolism. I. Effect of dietary cholesterol on the synthesis of cholesterol in dog tissue *in vitro. J. Biol. Chem.* **201:** 519–528.
33. Dietschy, J. M., and M. D. Siperstein. 1967. Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. *J. Lipid Res.* **8:** 97–104.