Cholesterol dynamics in rats fed *cis*- and *trans*-octadecenoate in the form of triglyceride

Michihiro Sugano, Kikuko Ryu, and Takashi Ide

Laboratory of Nutrition Chemistry, Kyushu University School of Agriculture, Fukuoka 812, Japan

Abstract The cholesterol dynamics were compared in rats fed diets containing either camellia oil or partially hydrogenated corn oil as a source of *cis*- and *trans*-octadecenoate, respectively. The diets contained approximately the same amount of octadecenoic acid, and an equivalent amount of linoleic acid. In rats fed the *trans*-fat for about 30 days, liver cholesterol levels were clearly low relative to levels in rats fed the *cis*-fat, while the concentration of serum cholesterol and the distribution of cholesterol in serum lipoproteins were comparable. The activity of hepatic microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase and the incorporation of [1-^{14}C]acetate into digitonin-precipitable sterols in the liver homogenate tended to increase in rats fed the *trans*-fat diet. Cholesterol 7α-hydroxylase activity increased significantly. Cholesterol absorption measured by the dual isotope serum ratio method was markedly lower, and much more radioactivity both from orally and intravenously administered cholesterol was excreted quickly into feces in the *trans*-fat group, with relatively more excretion in the neutral than in the acidic steroids. Apparent absorption of dietary fat was slightly lower in the *trans*-fat group. Cholesterol turnover as analyzed according to the two-pool model was much faster in rats fed *trans*-fat and the pool A size was reduced mainly as a consequence of stimulation of the removal rate from this pool. The intestinal epithelial cells contained relatively more *trans*-octadecenoate compared to the serum and liver and *trans*-fat modified the lipid composition specifically. These observations suggest that the changes in cholesterol metabolism due to the ingestion of *trans*-fat, compared to *cis*-fat, are as a result of metabolic events in the intestine.—**Sugano, M., K. Ryu, and T. Ide.** Cholesterol dynamics in rats fed *cis*- and *trans*-octadecenoate in the form of triglyceride. *J. Lipid Res.* **25:** 474–485.

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Although there is a trend toward an increase of partially hydrogenated vegetable oils into our food, consideration of the biological implications of *trans*-fatty acids seems insufficient, and a controversy still exists (1–3). The effect that *trans*-fatty acids exert on plasma cholesterol levels and hence atherosclerosis is, for example, still the subject of much debate (4–6); *trans*-fatty acids are hypercholesterolemic in certain cases or normocholesterolemic in others. The major reason for this discrepancy seems to be attributable to the differences not only in the types and amounts of *trans*-fatty acids ingested, but also to the amount of dietary linoleic acid in each experiment. In addition, the saturated and *cis*-fat components of dietary fat interact with the *trans*-fat component to determine the magnitude of the various responses (7).

Recent interest in the biological effect of *trans*-fatty acids has mainly been focused on elaidic (9-*trans*-octadecenoate) and linolelaidic acid (9-*trans*, 12-*trans*-octadecadienoate). However, the prototype of the *trans*-isomers in partially hydrogenated vegetable oils is *trans*-octadecenoate with double bonds not confined to a specific position (8), and elaidic acid usually represents only a relatively small portion of *trans*-fatty acids found in these products, since hydrogenation results in the migration of double bonds (1, 8). The metabolism of unsaturated fatty acids appears to be dependent on the position of double bonds (9). Although the *trans*-isomer of linoleic acid has attracted much attention because of its specific effect on the synthesis of prostaglandins and related physiologically active substances (2), this type of *trans*-fatty acid is usually a minor component in commercially available hydrogenated fats (10–13).

Thus it is important to examine the biological consequence of feeding *trans*-octadecenoate in the form of commercially available fats relative to the *cis*-counterpart. In the present study, the effects of *trans*-fat on cholesterol metabolism were compared in rats fed semipurified diets containing either camellia oil or partially hydrogenated corn oil. Corn oil is one of the vegetable oils currently being used for production of margarine. The diets contained approximately the same amount of *cis*- or *trans*-octa-decenoate. There was adequate linoleic acid in each diet, equivalent to 4% of the calories. The results showed specific biological effects of *trans*-octadecenoate in various aspects of cholesterol metabolism.

METHODS

Animals and diets

Specific-pathogen-free male Sprague-Dawley rats (Seiwa Experimental Animals, Ltd., Fukuoka) initially weighing

about 100 g were used throughout. The animals were housed individually in an air-conditioned room (22–25°C, lights on from 8 AM to 8 PM) and were fed experimental diets (**Table 1**) ad libitum (14, 15). Dietary fats used were commercial camellia oil (Yamakei Sangyo Co., Osaka) for a source of *cis*-octadecenoate and partially hydrogenated corn oil (16) for a source of the *trans*-counterpart. Food consumption and body weight were recorded three times each week. Three sets of experiments were performed. In the first experiment (Exp. 1), rats were killed by decapitation at midnight of days 30–32 for determination of liver enzyme activities and tissue lipids. Similar assays were made in rats killed in the morning of day 35 in the second experiment (Exp. 2). In the third experiment (Exp. 3), cholesterol absorption and turnover were measured after feeding experimental diets for 14 days.

Hepatic enzyme activity assays

The activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.1.34) in the liver (17) and intestine (18) was determined as described elsewhere. Cholesterol 7α-hydroxylase (EC 1.14.13.17) activity in the liver microsomes was determined by the method of Van Cantfort, Renson, and Gielen (19) using [4-^{14}C]cholesterol as a substrate. The incorporation of [1-^{14}C]acetate (Amersham International, Buckinghamshire, U.K.) into digitonin-precipitable sterols and fatty acids was measured in the 10,000 *g* supernatant (15 min) of the liver homogenate (20, 21). Microsomal protein was determined by the method of Lowry et al. (22).

TABLE 1. Compositions of experimental diets

Ingredients	*cis*-Fat	*trans*-Fat
	%	
Casein	20	20
Camellia oil[a]	8.35	0
Partially hydrogenated corn oil	0	7.60
Safflower oil	1.65	2.40
Mineral mixture[b]	4	4
Vitamin mixture[b]		
Water soluble	1	1
Fat soluble	0.1	0.1
Choline chloride	0.15	0.15
Cellulose powder	2	2
Sucrose	62.75	62.75
Fatty acid composition[c]	%	
16:0	8.3	10.6
18:0	2.1	7.7
t-18:1	trace	24.9
c-18:1	69.1	35.9
cc-18:2	19.9	19.9

[a] Camellia oil was purchased in one lot from a chemical distributor. Partially hydrogenated corn oil was prepared in one lot from commercial corn oil in the presence of stabilized nickel catalyst at a hydrogen pressure of 3 kg/cm^2 at 180–200°C (16).

[b] The mineral and water-soluble vitamin mixtures are according to Harpar (14). The fat soluble vitamins were dissolved in the corn oil.

[c] Fatty acid compositions were analyzed by GLC as described in the text.

Cholesterol absorption and turnover

Cholesterol absorption was measured by the dual isotope serum ratio method of Zilversmit and Hughes (23). After being fed the experimental diets for 14 days, rats were fasted for 12 hr and then were given intragastric [1,2(n)-^{3}H]cholesterol (17.8 μCi/0.5 ml, Amersham International) and intravenous [4-^{14}C]cholesterol (1.0 μCi/0.2 ml, New England Nuclear, Boston, MA) under light ether anesthesia. The labeled cholesterol was purified by thin-layer chromatography and dispersed in physiological saline containing 5% ethanol (24). Six hr after the administration of the cholesterol the diets were given freely. A small volume of blood was withdrawn from the tail vein on days 1, 2, 3, 5, 7, and 10 and then every 5 days up to 30 days for determination of radioactivity and enzymatic analysis of cholesterol (Cholesterol C-Test, Wako Pure Chemicals, Inc., Osaka). Feces were collected daily and lyophilized. On day 31 the rats were bled from the abdominal aorta after an overnight fast. Serum lipoproteins were separated by sequential ultracentrifugation in a Beckman 40.3 rotor (25). The die-away curves of the specific activity of serum cholesterol as a function of time were analyzed according to the two-pool model (26, 27) using the least-square, nonlinear regression method.

Apparent absorption of dietary fat

Fecal fat content was determined and the apparent rate of absorption of dietary fat was calculated by the method of van de Kamer, Huinink, and Weyers (28).

Radioactivity measurements

Radioactivity in the tissue lipid extracts was measured in an Aloka LSC-9000 liquid scintillation counter. The samples were dissolved in a toluene scintillator (0.2% PPO and 0.05% POPOP). Appropriate corrections for quenching were made. Feces were extracted with a mixture of chloroform–methanol 2:1 (v/v) at 50°C for 4 hr (29) and aliquots were taken for radioactivity measurements. Decolorization was found to be unnecessary. Other aliquots were separated into neutral and acidic steroid fractions by thin-layer chromatography (30). Bands corresponding to neutral steroids were scraped into counting vials and radioactivity was measured. Acidic steroids were extracted from the gel with chloroform–methanol mixture 2:1 (v/v) at 40°C for 30 min. The extract was dried and the radioactivity was measured in a methanol–toluene 1:9 (v/v) scintillation solution. Heart and adipose tissue were treated with 50% KOH at 90°C. Ethanol was added and lipids were saponified at 50°C for 1 hr. The unsaponifiable matter was then extracted with petroleum

ether, washed with water, and the radioactivity was measured.

Lipid analyses

Serum, liver, and intestinal mucosal lipids were extracted and purified according to Folch, Lees, and Sloane Stanley (31). Cholesterol, triglyceride, and phospholipid were determined as described elsewhere (32).

Fatty acid analyses

Liver microsomal lipids were extracted (31) and phosphatidylcholine and phosphatidylethanolamine were separated by thin-layer chromatography on silica gel G (33). Fatty acids liberated from phospholipids were methylated with diazomethane in diethyl ether (34). The resulting fatty acid methyl esters were analyzed by gas–liquid chromatography in Shimadzu 4CMPF and 8APF chromatographs equipped with a flame ionization detector. For separation of the geometric isomers, a glass column (6 m × 3 mm i.d.) packed with 15% OV-275 on 100/120 mesh Chromosorb PAW/DMCS was used (13, 16). Fatty acid methyl esters were also analyzed using 10% DEGS (containing 1% H_3PO_4) on Uniport HP (2 m × 3 mm i.d. glass column). These packings were purchased from Gaschro Kogyo Inc., Tokyo. The columns were standardized with purified fatty acid standards (Nu-Chek Prep Inc., Elysian, MN). Intestinal mucosal cells from rats in the second experiment were separated by scraping (18) and their fatty acid compositions were determined as described above. The mucosal lipids were also separated into triglyceride and phosphatidylcholine by thin-layer chromatography (33) and their fatty acid compositions were analyzed.

Histological examination

In the second experiment, upper and lower portions of the small intestine were stained with hematoxylin-eosin and examined by light microscopy.

Statistical analysis

The data were analyzed by Student's *t*-test (35).

RESULTS

Growth parameters and liver weight

Table 2 shows the growth parameters and liver weights. In each trial weight gain and relative liver weight were virtually the same in the *cis*- and *trans*-fat groups. Food intake was slightly but significantly greater in rats fed *trans*-fat than in the animals fed *cis*-fat in two out of three trials.

Serum and liver lipids

As **Table 3** shows, concentrations of serum cholesterol and phospholipid were comparable in the two fat groups in each of three experiments. Concentrations of triglyceride were also comparable in each paired group except for a large but statistically insignificant difference in rats killed at midnight (Exp. 1). The distribution of lipids in each lipoprotein fraction resembled each other except for a difference in LDL triglyceride. In experiment 3 blood was withdrawn at various time intervals in order to determine the specific activity of cholesterol in the nonfasting state. The serum cholesterol levels of these specimens were maintained at about 100 mg/dl in both groups of rats. Thus, comparing the fasting values of 70

TABLE 2. Effect of dietary fat on food intake, growth and liver weight[a]

Diet (No. of Rats)	Food Intake	Body Weight Gain[b]	Relative Liver Weight
	g/day	*g*	*g/100 g body wt*
Exp. 1			
cis-Fat (6)	16.2 ± 0.2[c]	201 ± 4	4.28 ± 0.13
trans-Fat (6)	17.3 ± 0.4	212 ± 7	4.48 ± 0.10
Exp. 2			
cis-Fat (8)	19.5 ± 0.5	249 ± 6	4.89 ± 0.19
trans-Fat (7)	19.3 ± 0.6	254 ± 10	5.15 ± 0.09
Exp. 3			
cis-Fat (8)	17.4 ± 0.5[c]	291 ± 6	3.87 ± 0.07
trans-Fat (8)	19.0 ± 0.3	303 ± 7	3.89 ± 0.07

[a] Results expressed as mean ± SE. Numbers of rats are in parentheses.
[b] Average initial body weights were 112, 105, and 91 g for experiments 1, 2, and 3, respectively. In Exp. 1, rats were fed diets for 30 to 32 days and killed at midnight. In Exp. 2, rats were fed for 36 days and killed in the morning. In Exp. 3, rats were fed for 46 days and killed after an overnight fast.
[c] Significant difference from the corresponding *trans*-group at $P < 0.05$.

TABLE 3. Effect of dietary fat on concentrations of serum lipids[a]

Diet (No. of Rats)	Serum Cholesterol	Serum Triglyceride	Serum Phospholipid
		mg/dl	
Exp. 1			
cis-Fat (6)	64.6 ± 2.9	553 ± 83	162 ± 2
trans-Fat (6)	71.0 ± 6.6	343 ± 49	165 ± 2
Exp. 2			
cis-Fat (8)	56.1 ± 1.7	30.2 ± 1.3	124 ± 5
trans-Fat (7)	58.6 ± 2.0	32.8 ± 3.2	126 ± 6
Exp. 3			
cis-Fat (8)	72.2 ± 0.3	35.8 ± 3.3	117 ± 14
VLDL[b]	(7.5 ± 1.1)[c]	(74.7 ± 2.7)	(5.9 ± 1.4)
LDL	(13.0 ± 1.9)	(7.8 ± 1.0)[d]	(15.7 ± 1.8)
HDL	(80.1 ± 1.8)	(17.5 ± 1.9)	(78.3 ± 2.1)
trans-Fat (8)	69.9 ± 2.7	41.4 ± 4.5	139 ± 23
VLDL	(8.0 ± 1.6)	(81.4 ± 2.4)	(7.6 ± 0.8)
LDL	(9.3 ± 0.7)	(3.6 ± 0.4)	(14.9 ± 3.1)
HDL	(82.6 ± 1.8)	(15.0 ± 2.4)	(77.5 ± 2.9)

[a] Results expressed as mean ± SE. For feeding conditions, see footnote to Table 2.
[b] Serum lipoproteins were separated by ultracentrifugation (25) into VLDL ($d < 1.006$ g/ml), LDL ($1.006 < d < 1.063$ g/ml) and HDL ($1.063 < d < 1.21$ g/ml).
[c] Percentage distribution of lipids in each fraction is in parentheses.
[d] Significant difference from the corresponding *trans* group at $P < 0.05$.

mg/dl in the same rats, there was a 30% reduction of serum cholesterol after overnight fasting.

The liver lipid data are summarized in **Table 4.** Livers from rats fed *trans*-fat contained less cholesterol and triglyceride than livers from animals fed *cis*-fat in all experiments, while phospholipid levels were comparable.

Cholesterol synthesis and oxidation

Effects of dietary fat types on several parameters of hepatic cholesterol synthesis were measured at the peak stage of diurnal rhythm (midnight) (Exp. 1) and the data are shown in **Table 5.** The specific activity of HMG-CoA reductase tended to be higher on feeding *trans*-fat. It has been reported that feeding rats a semipurified diet, compared to a nonpurified diet (commercial chow), results in a marked reduction of hepatic HMG-CoA reductase activity (36). The enzyme activities observed in the present study were comparable to those reported previously (37). The concentration of microsomal cholesterol was the same in rats fed *cis*- and *trans*-fat. The incorporation of [1-^{14}C]acetate into digitonin-precipitable sterols in the liver homogenate was significantly higher in rats fed *trans*-fat as compared to rats fed *cis*-fat. The activity of intestinal HMG-CoA reductase was also slightly higher both in the

TABLE 4. Effect of dietary fat on concentrations of liver lipids[a]

Diet (No. of Rats)	Liver Cholesterol	Liver Triglyceride	Liver Phospholipid
		mg/g wet wt	
Exp. 1			
cis-Fat (6)	3.01 ± 0.10[b]	11.8 ± 0.8[c]	34.5 ± 1.2
trans-Fat (6)	2.57 ± 0.07	7.14 ± 0.7	34.6 ± 0.7
Exp. 2			
cis-Fat (8)	2.82 ± 0.14[b]	20.9 ± 2.0	30.9 ± 0.6
trans-Fat (7)	2.07 ± 0.14	14.4 ± 1.6	32.0 ± 1.6
Exp. 3			
cis-Fat (8)	4.29 ± 0.11[c]	21.2 ± 3.0	25.8 ± 1.6
trans-Fat (8)	2.61 ± 0.20	15.4 ± 1.7	24.5 ± 1.8

[a] Results expressed as mean ± SE. For feeding conditions, see footnote to Table 2.
[b] Significant difference from the corresponding *trans* group at $P < 0.05$.
[c] Significant difference from the corresponding *trans* group at $P < 0.01$.

TABLE 5. Effect of dietary fat on cholesterol synthesis and oxidation[a]

Diet	Liver					Small Intestine	
	Microsomal HMG-CoA Reductase	Microsomal Cholesterol 7α-Hydroxylase	[1-^{14}C]Acetate Incorporation into[b]		Microsomal Cholesterol	HMG-CoA Reductase	
			DPS	FA		Proximal	Distal
	pmol/min per mg protein		*nmol/hr per g liver*		*μg/mg protein*	*pmol/min per mg protein*	
cis-Fat	51.9 ± 5.4	3.80 ± 0.54[c]	1.35 ± 0.15[d]	72.2 ± 7.0	21.2 ± 3.6	293 ± 76	15.7 ± 6.6
trans-Fat	72.3 ± 8.9	7.39 ± 0.53	1.89 ± 0.15	85.2 ± 23.5	21.2 ± 1.7	335 ± 135	25.0 ± 6.6

[a] Results expressed as mean ± SE for six rats in each dietary group (Exp. 1). Rats were fed these diets for 30 to 32 days and killed at midnight.
[b] Acetate incorporation in the liver homogenate (10,000 *g*, 15 min supernatant). DPS, digitonin-precipitable sterols; FA, fatty acids.
[c] Significant difference from the *trans* group at $P < 0.01$.
[d] Significant difference from the *trans* group at $P < 0.05$.

proximal and distal portions of the intestine from rats fed a *trans*-fat diet. These rats also exhibited a significantly greater specific activity of microsomal cholesterol 7α-hydroxylase.

Hepatic HMG-CoA reductase activity in the *trans*-fat group was significantly higher during diurnal nadir stage (in the morning) (Exp. 2) than in the *cis*-fat group (34.8 ± 6.1 vs. 13.2 ± 1.8 pmol/min per mg protein, $P < 0.01$).

Absorption of cholesterol and dietary fat

Except for day 1, for which the results were somewhat variable, the rate of cholesterol absorption calculated by the ratio of two different isotopes in the serum was constant for at least 10 days. The rate declined very slightly thereafter but the relative ratio between the two groups was nevertheless kept constant. Therefore, the cholesterol absorption rate was calculated during days 2 to 10 and the data were pooled and averaged. As shown in **Table 6,** the cholesterol absorption rate was markedly lower in rats fed *trans*-fat than in those fed *cis*-fat. In addition, the apparent absorption rate of partially hydrogenated corn oil was slightly but significantly lower as compared to that of camellia oil.

TABLE 6. Absorption of cholesterol and fat[a]

Diet	Cholesterol Absorption[b]	Dietary Fat Absorption[c]
	%	
cis-Fat	70.6 ± 2.6[d]	98.9 ± 0.2[d]
trans-Fat	53.8 ± 2.2	94.9 ± 0.4

[a] Results expressed as mean ± SE for eight rats in each dietary group (Exp. 3).
[b] Cholesterol absorption rate was measured by the dual isotope ratio method (23). The absorption rate was calculated for 2 to 10 days after administration of labeled cholesterol. The data were pooled and averaged. For details, see the text.
[c] Apparent absorption rate was measured by the method of van de Kamer, Huinink, and Weyers (28).
[d] Significant difference from the *trans* group at $P < 0.01$.

Turnover rate of cholesterol

Fig. 1 illustrates the time course of serum cholesterol specific activity. The decay of cholesterol specific activity in the serum of rats fed a *trans*-fat diet was more rapid than in rats fed a *cis*-fat diet. The half-life which was calculated at the equilibrated phase was significantly shorter on feeding *trans*-fat ($t_{1/2B}$ 18.2 ± 1.2 vs. 13.6 ± 1.1 days, $P < 0.05$). The curves were found to confirm most closely to a two-pool model as proposed by Goodman and Noble (26) and Nestel, Whyte, and Goodman (27). The kinetic parameters thus calculated are summarized in **Table 7.**

The cholesterol mass in the rapidly exchangeable pool (pool A) decreased significantly on feeding a *trans*-fat diet, being approximately two-thirds that of rats fed a *cis*-fat diet, while the estimated minimum masses of slowly exchangeable pool (pool B) were comparable. The metabolic turnover rate of cholesterol (PR_A) was slightly but significantly higher in rats fed a *trans*-fat diet relative to those fed a *cis*-fat diet. Although the rate constant for the transfer rate of cholesterol from pool A to pool B (K_{AB}) remained unchanged, that for the removal rate of cholesterol from pool A (K_A) was doubled in rats fed a *trans*-fat diet. Consequently, the rate constant for the total rate of the removal of cholesterol from pool A (K_{AA}) was considerably but insignificantly high in rats fed a *trans*-fat (−0.47 ± 0.08 vs. −0.31 ± 0.04 per day).

Excretion of cholesterol and bile acids

Fig. 2 shows a time course of cumulative radioactivity excretion into feces of rats fed experimental diets for 14 days prior to receiving radioactive cholesterol. Feeding of *trans*-fat resulted in a marked stimulation of the excretion of radioactivity from both orally and intravenously administered cholesterol, although the slopes of the ex-

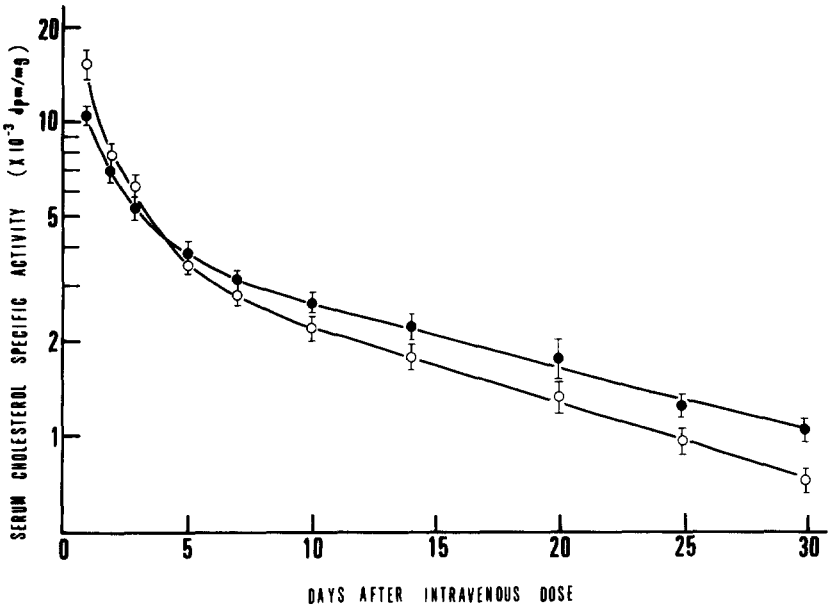

Fig. 1. Decay of serum cholesterol specific activity following intravenous injection of [4-^{14}C]cholesterol to rats fed diets containing either camellia oil (*cis*-fat) or partially hydrogenated corn oil (*trans*-fat). Values are expressed as mean ± SE for seven and six rats in *cis*- and *trans*-fat groups (Exp. 3), respectively; *cis*-fat, — ● —; *trans*-fat, — ○ —.

cretion curves differed somewhat. As **Fig. 3** shows, the extent of an increase in the fecal radioactivity was much more marked in the neutral steroid fraction than in the acidic steroid fraction after intragastric administration of labeled cholesterol.

Radioactivity remaining in tissues

Thirty days after the dual doses of radioactive cholesterol, rats were fasted overnight and killed. The liver, heart, and epididymal adipose tissue were excised, and the radioactivity in these tissues was determined. Rats fed *trans*-fat contained much less radioactivity from both orally and intravenously administered cholesterol compared to rats fed the *cis*-fat diet. The extent of the difference was most marked in the liver (as % of dose, 3.98 ± 0.46 vs. 0.95 ± 0.11, $P < 0.01$ and 6.11 ± 0.75 vs. 1.95 ± 0.26, $P < 0.01$ for oral and intravenous cholesterol, respectively).

Fatty acid compositions of liver and intestine

The fatty acid compositions of microsomal phosphatidylcholine and phosphatidylethanolamine isolated from the livers from rats fed diets for about 30 days (Exp. 1) are summarized in **Table 8.** Although there were some differences in fatty acid compositions of these phospholipids in the two groups, the percentages of linoleic and arachidonic acids were not altered in each phospholipid. The extent of incorporation of *trans*-octadecenoate was the same in these two phospholipids.

Table 9 shows the fatty acid compositions of intestinal mucosa of rats fed experimental diets for about 35 days and killed in the morning (Exp. 2). Total lipids from both

TABLE 7. Effect of dietary fat on kinetic parameters of cholesterol turnover[a]

Diet	M_A	PR_A	K_{AB}	K_A	M_B (min)
	mg	*mg/day*	*/day*	*/day*	*mg*
cis-Fat	164 ± 9[b]	18.3 ± 0.6[b]	0.20 ± 0.04	0.11 ± 0.01[b]	191 ± 8
trans-Fat	116 ± 13	21.6 ± 0.8	0.27 ± 0.05	0.20 ± 0.02	220 ± 17

[a] Results expressed as mean ± SE for six and seven rats in *cis*- and *trans*-fat groups, respectively (Exp. 3). Kinetic parameters were calculated according to the two-pool model (26, 27). M_A, mass of cholesterol in pool A; PR_A, the metabolic turnover rate of cholesterol; K_{AB}, the rate constant for the rate of transfer of cholesterol from pool A to pool B; K_A, the rate constant for the rate of removal of cholesterol from pool A; and M_B, the minimum mass of cholesterol in pool B, assuming that cholesterol synthesis in tissues of pool B is negligible.

[b] Significant difference from the *trans* group at $P < 0.05$.

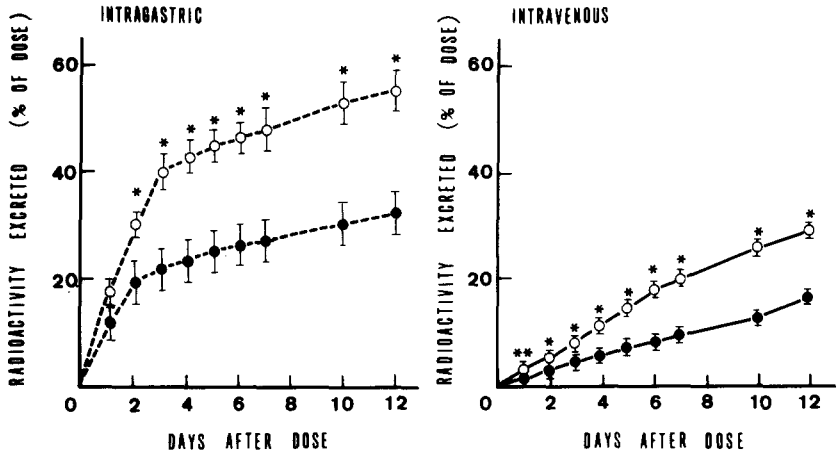

Fig. 2. Cumulative excretion of radioactivity into feces following administration of intragastric [1,2-^{3}H]cholesterol and intravenous [4-^{14}C]cholesterol to rats fed diets containing either camellia oil (*cis*-fat) or partially hydrogenated corn oil (*trans*-fat). Values are expressed as mean ± SE for eight rats in each dietary group (Exp. 3). *cis*-fat, — ● —; *trans*-fat, — ○ —. *,**Significant difference from the *cis*-fat group at $P < 0.01$ or 0.05, respectively.

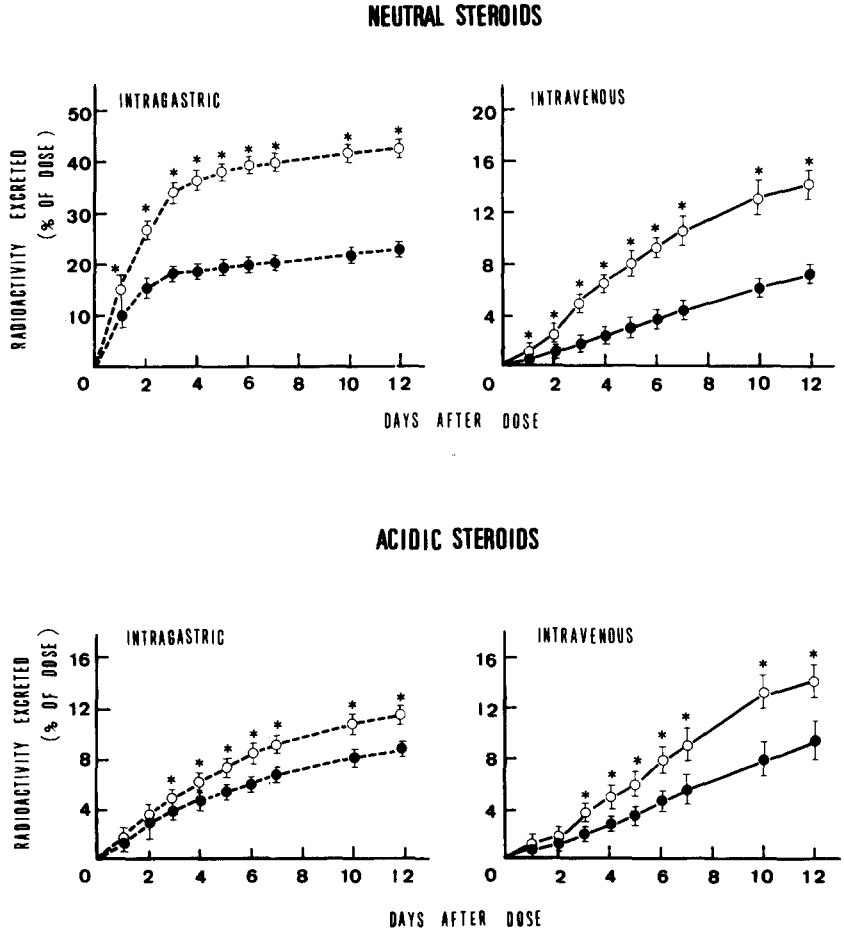

Fig. 3. Cumulative excretion of radioactivity into fecal neutral and acidic steroid fractions following administration of intragastric [1,2-^{3}H]cholesterol and intravenous [4-^{14}C]cholesterol to rats fed diets containing either camellia oil (*cis*-fat) or partially hydrogenated corn oil (*trans*-fat). Values are expressed as mean ± SE for eight rats in each dietary group (Exp. 3). *cis*-fat, — ● —; *trans*-fat, — ○ —. *Significant difference from the *cis*-fat group at $P < 0.01$.

TABLE 8. Effect of dietary fat on fatty acid composition of hepatic phospholipids[a]

Diet (No. of Rats)	Fatty Acid								
	16:0	*t*-16:1	*c*-16:1	18:0	*t*-18:1	*c*-18:1	*tc*-18:2	*cc*-18:2	20:4	22:6
	weight %									
Liver microsome										
Phosphatidylcholine										
cis-Fat (6)	25.8 ± 1.6	tr	1.7 ± 0.1	24.6 ± 1.4[b]	tr	15.6 ± 0.5[b]	tr	9.2 ± 0.7	17.9 ± 1.5	1.5 ± 0.2
trans-Fat (6)	22.7 ± 0.4	0.6 ± 0.1	1.3 ± 0.2	19.5 ± 0.6	9.7 ± 0.6	11.6 ± 0.3	0.6 ± 0.1	11.0 ± 0.8	18.6 ± 0.7	1.0 ± 0.1
Phosphatidylethanolamine										
cis-Fat (6)	22.3 ± 1.1	tr	1.1 ± 0.0	28.4 ± 1.3	tr	9.9 ± 0.9[c]	tr	5.2 ± 0.7	24.6 ± 1.1	4.4 ± 0.5
trans-Fat (6)	19.9 ± 1.5	0.4 ± 0.2	0.9 ± 0.0	22.0 ± 0.8[c]	9.5 ± 1.0	7.0 ± 0.3	0.4 ± 0.2	5.9 ± 0.8	25.2 ± 1.3	3.3 ± 0.5

[a] Results expressed as mean ± SE (Exp. 1). Fatty acid compositions were determined by GLC using a combination of DEGS and OV-275 columns as described in the text. tr, trace.
[b] Significant difference from the *trans* group at $P < 0.01$.
[c] Significant difference from the *trans* group at $P < 0.05$.

the upper jejunum and lower ileum contained much more *trans*-octadecenoate (14–16%) than did the serum and liver (8–10%). In addition, rats fed the *trans*-fat had higher percentages of polyunsaturated fatty acids, in particular arachidonic acid as compared to rats fed the *cis*-fat. Upper jejunal lipids were separated into triglyceride and phosphatidylcholine and their fatty acid compositions were analyzed. There was relatively more *trans*-octadecenoate in triglyceride relative to phospholipid, while changes in the percentages of arachidonic acid were confined exclusively to phosphatidylcholine.

Lipid compositions and histology of intestinal mucosa

The lipid compositions of intestinal mucosa (Exp. 2) are summarized in **Table 10.** There were considerable dietary fat-dependent differences in concentrations of lipids. Rats fed the *trans*-fat contained significantly more cholesterol and phospholipids and less triglyceride.

In some specimens examined, the height of villi in rats fed a *trans*-fat diet tended to be longer both in the proximal and distal intestine (approximately 10–20%) than in the animals fed a *cis*-fat diet (Exp. 2). No other structural differences were obvious in the microscopic field.

DISCUSSION

Emken (38) has summarized studies on the effect of elaidic acid of hydrogenated fat on serum lipids in human subjects and emphasized the divergent results and the lack of consensus. The same situation seems to be the case for the experimental animals (4, 5, 39). In these latter studies, it appears that the critical variable in diets was the amount of linoleic acid, inasmuch as *trans*-fatty acids aggravate essential fatty acid deficiency (2, 40). In our studies with Wistar rats, *trans*-octadecenoate supplied as partially hydrogenated soybean oil was more cholesterolemic than the untreated soybean oil, particularly when cholesterol-enriched diets were fed (16). However, the contents of linoleic and saturated fatty acids were very different in these diets. It is well known that saturated fat is twice as important, gram for gram, in its effect on elevating plasma cholesterol as is polyunsaturated fat. When *trans*-octadecenoate (as partially hydrogenated corn oil) was compared to *cis*-octadecenoate (as olive oil), there were no differences in serum cholesterol levels irrespective of the presence or absence of dietary cholesterol when the amounts of dietary linoleic acid were equivalent to each other; in some instances the *trans*-acid was hypocholesterolemic (14, 15). Moore, Alfin-Slater, and Aftergood (41) also recently reported in their long term study with rats that serum cholesterol levels were decreased when animals were fed partially hydrogenated fat containing various amounts of *trans*-fatty acids when

TABLE 9. Effect of dietary fat on fatty acid composition of intestinal lipids[a]

Diet (No. of Rats)	Fatty Acid								
	16:0	*t*-16:1	*c*-16:1	18:0	*t*-18:1	*c*-18:1	*tc*-18:2	*cc*-18:2	20:4
	weight %								
Upper jejunum									
Total lipids									
cis-Fat (8)	15.7 ± 0.8	tr	2.8 ± 0.4	8.2 ± 1.0	tr	52.2 ± 1.7[b]	tr	13.4 ± 0.8[b]	5.0 ± 0.8[b]
trans-Fat (7)	15.7 ± 0.8	0.6 ± 0.1	2.2 ± 0.3	10.3 ± 0.7	15.8 ± 0.8	25.6 ± 1.0	1.4 ± 1.0	16.9 ± 0.4	8.1 ± 0.8
Triglyceride									
cis-Fat (7)	18.7 ± 0.4	tr	4.2 ± 0.5	2.5 ± 0.5[b]	tr	63.3 ± 0.5[b]	tr	9.1 ± 0.4[b]	0.2 ± 0.1[c]
trans-Fat (7)	20.5 ± 0.8	0.7 ± 0.1	3.6 ± 0.3	5.6 ± 0.4	19.5 ± 1.4	34.1 ± 1.0	0.9 ± 0.1	11.6 ± 0.5	0.5 ± 0.1
Phosphatidylcholine									
cis-Fat (7)	21.7 ± 0.7	tr	2.6 ± 0.2[b]	16.2 ± 1.5	tr	37.6 ± 3.8[b]	tr	14.0 ± 1.1[b]	4.3 ± 0.8[b]
trans-Fat (7)	21.7 ± 1.0	1.0 ± 0.1	1.1 ± 0.1	15.4 ± 0.4	9.8 ± 0.9	17.9 ± 0.7	0.3 ± 0.1	22.5 ± 1.0	7.3 ± 0.3
Lower Ileum									
Total Lipids									
cis-Fat (8)	18.2 ± 1.0	tr	3.6 ± 0.3	4.6 ± 0.7[c]	tr	57.3 ± 1.6[b]	tr	10.9 ± 0.3[c]	2.4 ± 0.8[b]
trans-Fat (7)	18.4 ± 0.8	0.9 ± 0.1	2.9 ± 0.4	8.4 ± 1.0	14.0 ± 0.8	28.4 ± 1.6	1.5 ± 0.1	12.3 ± 0.8	7.4 ± 1.2

[a] Results expressed as mean ± SE (Exp. 2). Fatty acid compositions were determined by GLC using a combination of DEGS and OV-275 columns as described in the text. tr, trace.
[b] Significant difference from the corresponding *trans* group at $P < 0.01$.
[c] Significant difference from the corresponding *trans* group at $P < 0.05$.

compared with the animals fed corn oil or lard. The present results confirm that in a different strain of rats, i.e., Sprague-Dawley, *trans*-octadecenoate did not elevate serum cholesterol under various nutritional conditions (Table 3). When dietary linoleic acid and saturated fatty acids are comparable, the *trans*-monoene in the form of triglyceride can at least be regarded non-hypercholesterolemic as compared to the *cis*-monoene.

Under dietary conditions nearly comparable to those of the present study, *trans*-octadecenoate was shown to increase fecal excretion of neutral and acidic steroids, particularly when diets free of cholesterol were fed to rats (14, 15). The present experiment certainly supports these observations. The fecal excretion of radioactivity from both orally and intravenously administered cholesterol was markedly higher on feeding a *trans*-fat diet (Fig. 2). The demonstration that the excretion was much greater in the neutral than in the acidic steroid fraction agrees with data obtained by chemical analyses (14, 15). There was also noted a markedly lower rate of cholesterol absorption in rats fed the *trans*-fat diet. Also, the slightly but significantly lower apparent absorption of *trans*-fat compared to *cis*-fat is consistent with the report by Danvillier et al. (42). The results suggest a possible association of a reduction of cholesterol absorption with a lower rate of fat absorption. It is well known that the higher the melting point of dietary fat, the lower its absorption. Possibly this is due to limited lipolysis and hence restricted solubilization in micelles (43), inasmuch as the extent of absorption of free elaidic acid and free oleic acid is similar (44). Fatty acids or monoglycerides enhance the solubilization of cholesterol in bile salt micelles and unabsorbed or undigested fat may restrict availability of cholesterol to the jejunal mucosa. In fact, absorption of cholesterol is restricted when it is ingested simultaneously with saturated fat compared to unsaturated fat (43). Thus, the effect of fat on cholesterol absorption is related to the degree of saturation. It has been suggested that *trans*-fatty acids resemble saturated fats in their metabolic action (3, 45). In addition, unhydrolyzed triglyceride inhibits taurocholic acid absorption in the distal small bowel (46).

In contrast, Feldman and Russell (47) have suggested that the poor fat absorption is not the entire explanation for lower cholesterol absorption. The possibility exists that the function of absorptive cells is modified by the type of dietary fat. Optical microscopic examination has shown, on occasion, a recognizable elongation of the up-

TABLE 10. Effect of dietary fat on concentrations of jejunal lipids[a]

Diet	Jejunal Cholesterol	Jejunal Triglyceride	Jejunal Phospholipid
	mg/g		
cis-Fat	2.05 ± 0.22[b]	18.3 ± 3.8[c]	3.80 ± 0.27[b]
trans-Fat	2.99 ± 0.18	8.3 ± 1.1	6.96 ± 0.85

[a] Results expressed as mean ± SE for 8 and 7 rats in *cis*- and *trans*-fat groups, respectively (Exp. 2). The mucosal cells were isolated by scraping from the upper portion of the jejunum.
[b] Significant difference from the *trans* group at $P < 0.01$.
[c] Significant difference from the *trans* group at $P < 0.05$.

per jejunal villi after feeding a *trans*-fat diet. The lipid composition of the absorptive cells was also modified by the type of dietary fat in the present study (Table 10). The fatty acid composition data indicated a relatively high rate of incorporation of dietary *trans*-fatty acid into the epithelial cells, as compared to incorporation into the serum and liver. Since rats in this experiment were killed in the morning, the possibility that ingested fat was still in the mucosa is remote; serum triglyceride levels were indeed low, together with a relatively low incorporation of *trans*-fatty acids. Additionally, differences in the percentages of polyunsaturated fatty acids were observed only in the intestinal mucosal cells. Feeding of *trans*-fat results in modulation of membrane fluidity of the mucosal cells as compared to *cis*-fat (45, 48), and hence may modify the uptake and further metabolism of cholesterol. The observation that the mucosal cells of the upper jejunum contained less triglyceride suggests changes in several parameters of triglyceride metabolism including absorption, resynthesis, and transport, although it is not clear which metabolic process is responsible for the modification of the triglyceride contents.

Kinetic parameters derived from the decay curves (Table 7) showed that diets containing *trans*-fat produced a significantly smaller mass of pool A while the estimated size of pool B remained apparently unchanged. The reduction of pool A mass seems to be principally attributable to reduction of the hepatic cholesterol pool, rather than to circulating cholesterol, the concentration of liver cholesterol was significantly lower in the *trans*-fat group (Table 4). A rise of liver cholesterol as a result of a *cis*-unsaturated fat diet relative to a saturated fat diet has been frequently reported (43, 47). The rate of removal of cholesterol from pool A was marked, suggesting that more cholesterol is lost via bile in rats fed *trans*-fat. This phenomenon is consistent with the increased rate of fecal excretion of steroids, confirmed both by chemical analysis (14, 15) or radiochemically (Fig. 2). In addition, the incorporation of *trans*-fatty acids into serum lipoproteins may influence removal mechanisms through a change in lipoprotein structure (49).

To maintain pool A mass under such circumstances, cholesterol input into this pool should be exaggerated. In fact, cholesterogenesis in the liver and intestine tended to increase on feeding *trans*-fat (Table 3). Alternatively, the rise of hepatic cholesterol synthesis could have resulted from less negative feedback inhibition (50), somewhat similar to the effect of sterol removal from the enterohepatic circulation by a solid triglyceride (17, 43, 44). The extent of enhancement of cholesterol 7α-hydroxylase, the key enzyme in bile acid synthesis from cholesterol, was more marked than that of cholesterol synthetic activity. Thus, the newly synthesized cholesterol seems to be preferentially removed as bile acids. Balasubramaniam, Mitropoulos, and Myant (51) confirmed such a mechanism in rats. Björkhem, Blomstrand, and Svensson (52) showed an inhibitory effect of triolein and trilinolein on 7α-hydroxylation of cholesterol as compared to tripalmitin and trierucin. The level of cholesterol 7α-hydroxylase activity was found to be better related to the degree of absorption of fat than to total amount of absorbed fat or the degree of unsaturation of the fat. Intestinal absorption of bile acids appears to be influenced by the type of dietary lipids (53). A large loss of bile acids in feces (14, 15) may then increase 7α-hydroxylation of cholesterol due to reduced feedback inhibition (54). From these results it seems likely that the removal of cholesterol from the liver outweighs the enhanced synthesis of cholesterol, thus causing a reduction of pool A mass in rats fed *trans*-fat. In swine, however, the specific activity of hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase was not influenced by dietary fat containing various proportions of *trans*-, *cis*-, and saturated fat (7). Ramasha, Paul, and Ganguly (55) showed a reduction of several parameters for cholesterol and bile acid synthesis by commercial hydrogenated vegetable oil compared to safflower oil, although no data for the *trans*-fatty acid content of the hydrogenated oil were given. These inconsistencies are almost exclusively ascribed to the experimental conditions where no appropriate controls were provided with respect to the degree of unsaturation and/or the supply of linoleic acid in the dietary fats.

The fatty acid composition of phospholipids in the organelles is of interest regarding their membrane function. There were no marked differences in compositions of microsomal phospholipids in the livers from rats fed the *trans*- or *cis*-fat except for the occurrence of *trans*-fatty acids in the former (Table 8). Both groups of rats contained similar amounts of physiologically active polyunsaturated fatty acids, thus reflecting a comparable status of essential fatty acids. *Trans*-fatty acids were presumed to be incorporated primarily into the 1-position of these glycerophospholipids (56), since the relative amounts of palmitic and stearic acids decreased with the incorporation of *trans*-fatty acids and since the decrease in percentage of oleic acid may be fully compensated by the partial replacement with *trans*-octadecenoate. Thus, the membrane function of the liver microsomes seems at least to be maintained unchanged, even in rats fed *trans*-fat relative to those fed *cis*-fat. The effect of *trans*-fatty acids on biological functions, if any, will disappear when the *trans*-fat diet is discontinued (57). We found[1] that dietary *trans*-octadecenoate, as compared to the *cis*-counterpart, had no effect on the activities of several mixed function oxidases including aminopyrine N-demethylase, *p*-aniline

[1] Sugano, M., K. Ryu, and T. Ide. Unpublished results.

hydroxylase, and biphenyl hydroxylase. Also, no difference was found in the activity of NADPH-cytochrome c reductase and the content of cytochrome P-450 in hepatic microsomes of rats fed *cis*- or *trans*-fat. Although the activity of cholesterol 7α-hydroxylase was elevated in rats fed the *trans*-fat, the activity was not correlated with the concentration of total cytochrome P-450 or with NADPH-cytochrome c reductase activity in liver microsomes in a variety of states in which the activity of the hydroxylase was altered (58). Ruttenberg et al. (39) were also unable to demonstrate significant differences in activities of hepatic microsomal, cytosolic, and mitochondrial enzymes in rabbits fed different levels of *trans*-octadecenoate.

In summary, the *trans*-octadecenoate even at a relatively higher dose in no way exhibited any untoward effects on cholesterol metabolism, especially insofar as the serum cholesterol level was concerned, as long as the supply of linoleic acid was adequate. Partially hydrogenated fats rich in this type of *trans*-monoene reduced cholesterol mass in the rapidly exchangeable pool without influencing the mass in slowly exchangeable pool. It is thus difficult to demonstrate an association between dietary *trans*-monoene and atherosclerosis. Studies of the influence of *trans*-fat on experimental atherosclerosis show, with one exception in swine (59), that it is no more atherogenic than *cis*-fat in swine (60) and rabbit (39, 61). Modulations of the intestinal cells to process cholesterol seem to be a primary factor for changes in cholesterol dynamics on feeding *trans*-octadecenoate as compared to the *cis*-isomer.

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