Dietary fiber decreases cholesterol and phospholipid synthesis in rat intestine[1]

Sheldon E. Schwartz,[2] Christopher Starr, Suzanne Bachman, and Philip G. Holtzapple

Department of Medicine, State University of New York, Upstate Medical Center, Syracuse, NY 13210

Abstract The effects of fiber ingestion on the incorporation of oleic acid into triglyceride and lecithin, acetate incorporation into cholesterol, and monosaccharide and amino acid transport were determined in rat intestine. Prolonged pectin (10% by weight) ingestion caused a decrease in jejunal and ileal cholesterol synthesis (33% and 52%, respectively). Pectin ingestion reduced cholesterol synthesis by 60% in ileal crypt cells, but did not affect cholesterol synthesis in the jejunal or ileal villus cells or in jejunal crypt cells. Cholesterol synthesis in isolated crypt cells was markedly less than in isolated villus cells. Prolonged ingestion of a fiber-free diet supplemented with either cellulose or pectin (10% and 5% by weight, respectively) decreased jejunal lecithin glucose and leucine absorption but did not affect jejunal triglyceride synthesis.—**Schwartz, S. E., C. Starr, S. Bachman, and P. G. Holtzapple.** Dietary fiber decreases cholesterol and phospholipid synthesis in rat intestine. *J. Lipid Res.* 1983. **24:** 746–752.

Supplementary key words triglyceride synthesis • intestinal absorption • cell membrane • isolated intestinal cells

Dietary fibers are substances derived from plants and they are not digested in the small intestine. In both animals and humans sustained ingestion of water-soluble dietary fibers is associated with a reduction in blood cholesterol and a blunted response in serum glucose after a carbohydrate meal (4–9). The reduction in blood cholesterol is purportedly related to the formation of intraluminal neutral and acidic sterol complexes resulting in an increase in fecal sterol excretion (10, 11). Altered glucose tolerance has been ascribed to an interference of glucose diffusion from the bulk phase to the intestinal membrane resulting in a decrease in the rate of glucose absorption (12, 13).

Recent work suggests that some of the physiological effects of fiber are not caused simply by intraluminal processes. Sustained supplementation with fiber alters the intestinal surface topography (14), impairs intestinal glucose transport (15), and modifies sodium and chloride fluxes in rat jejunal mucosa (16). These observations suggest that fiber interferes with normal absorptive mechanisms by altering the epithelial cell membrane or cellular metabolic events.

We studied the effects of prolonged fiber supplementation on: intestinal incorporation of oleic acid into triglyceride and lecithin, acetate incorporation into cholesterol, and transport of a monosaccharide and an amino acid (glucose and leucine). Our studies suggest that fiber ingestion affects lecithin and cholesterol synthesis and intestinal absorption. These observations cannot be explained by the intraluminal physicochemical properties of dietary fiber.

MATERIALS AND METHODS

Materials

Solvents for lipid extraction and chromatography were high purity glass-distilled reagents obtained from Burdick and Jackson Laboratories, Muskegon, MI; silica gel G (0.25 mm) 20 × 20 cm plates were obtained from Analtech Inc., Newark, DE. Cholesterol standards, reagents and standards for RNA, DNA, and protein determinations, and standards for disaccharidase and alkaline phosphatase activities were obtained from Sigma Chemical Co., St. Louis, MO. Phospholipid and triglyceride standards were obtained from Applied Science Laboratories, University Park, PA. Buffers were prepared using certified ACS reagents obtained from Sigma Chemical Co., St. Louis, MO and Fisher Scientific Co., Rochester, NY. NCS tissue solubilizer was obtained from Amersham-Searle Corp., Arlington Heights, IL. Radiochemicals including [1-^{14}C]oleic acid (58 mCi/mmol), [1-^{14}C]acetic acid-sodium salt (56.2 mCi/mmol), D-[^{14}C-U]glucose (13.9 mCi/mmol), L-[^{14}C-U]leucine (355 mCi/mmol), and [methoxy-^{3}H]inulin (250 mCi/g) were obtained from New England Nuclear Co., Boston, MA. Soy protein, Hegsted salt mixture, vitamin diet fortification mixture, alphacellulose (Alphacel), and cholesterol were obtained from ICN, Cleveland, OH.

Abbreviations: KRB, Krebs-Ringer bicarbonate buffer.

[1] This work has been published in abstract form (1–3).

[2] To whom reprint requests should be addressed at: Department of Medicine, State University of New York, Upstate Medical Center, 750 East Adams Street, Syracuse, NY 13210.

Pectin was obtained as apple pectin powder from Solgar Co., Lynbrook, NY. Cholestyramine was obtained as Questran from Mead Johnson Co., Evansville, IN.

Animals and diets

Male weanling Sprague-Dawley rats (50–70 g) were housed individually in cages with a wire-mesh bottom and placed in a constant temperature-humidity room with a 12-hr light–dark cycle. All animals were maintained on a special diet for a 6-wk experimental period, and all experiments were performed during the midportion of the dark cycle. One group of animals had continual access to a semisynthetic fiber-free diet consisting of 62% sucrose, 25% soy protein, 5% corn oil, 5% Hegsted salt mixture, and 4% vitamin diet fortification mixture. The other groups received the same diet to which either cellulose (10% by weight) or pectin (5% or 10% by weight) was added. Weight gain was not significantly different for rats in any of the groups (rats ingesting fiber-supplemented diets consumed more food). Animals maintained on the fiber-free diet for 6 wk weighed 317 ± 11 g (mean ± SEM), on the cellulose diet 292 ± 10 g, 304 ± 11 g on the 5% pectin diet, and 314 ± 12 g on the 10% pectin diet.

After ingesting the diets for the 6-wk period, rats were fasted (allowed access to water) for 18 hr, weighed, stunned, and killed by cervical dislocation. Blood was obtained for serum cholesterol determinations. All isolation experiments were performed in a cold room at 4°C. Tissue was maintained in cold phosphate-buffered saline (pH 7.4).

Cholesterol synthesis in everted intestinal sacs

Duplicate everted proximal jejunal and distal ileal sacs were prepared from rats maintained for 6 wk on the fiber-free diet (see above) or on the fiber-free diet to which 10% pectin, 0.7% cholesterol, 0.7% cholesterol and 10% pectin, or 2.5% cholestyramine was added. Sacs were placed in vials containing 2 ml of Krebs-Ringer bicarbonate buffer (KRB) saturated with 95% O_2:5% Co_2 (pH 7.4), 5 mM glucose, and [1-^{14}C]acetate (1 μCi/ml). The solutions were oxygenated throughout the incubation period. After a 30-min incubation at 37°C in a metabolic shaker, sacs were rinsed in cold buffer, emptied of their serosal fluid, immersed in cold acid saline (pH 2.0), and homogenized. Four volumes of ethanolic KOH were added to an aliquot of the homogenate and saponified for 2 hr at 80°C. Total cholesterol was extracted with petroleum ether, and the extracts were chromatographed on activated silica gel G plates developed with petroleum ether–diethylether–acetic acid 136:64:0.4. Areas corresponding to cholesterol were scraped into vials to which scintillation fluid was added. Radioactivity was measured using an external standard to correct for quenching. Cholesterol synthesis is expressed as dpm of [1-^{14}C]acetate incorporated per 30 min per mg protein (dpm/30 min per mg protein). No cholesterol was noted in the serosal fluid after a 30-min incubation.

In validation experiments, the area corresponding to cholesterol was scraped, eluted with chloroform, and dried under nitrogen. Cholesterol was identified by gas–liquid chromatography on a (Model 5830A, Hewlett-Packard Co., Cupertino, CA) equipped with a flame ionization detector and a siliconized glass column (2 mm × 2 mm i.d.) packed with 3% OV-17 coated on 100/200 mesh Gas-chrome Q (Applied Science Laboratories). Retention for cholesterol was 12 min and a single peak was observed. Extraction and plating efficiency of [1,2-^{3}H(N)]cholesterol exceeded 95%.

Cholesterol synthesis in isolated intestinal cells

Isolated cells (17) were obtained from rats maintained for 6 wk on either the fiber-free diet or the 10% pectin diet. A 20-cm segment of proximal jejunum and distal ileum were excised from each animal and rinsed with ice-cold 154 mM NaCl. Each intestinal loop was ligated at one end and filled with oxygenated buffer A (pH 7.2) containing 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, and 10 mM glucose. After closure of the other end, each loop was immersed in continuously oxygenated 154 mM NaCl and gently agitated in a metabolic shaker. At the conclusion of a 20-min incubation at 37°C, buffer A was decanted and the segments were filled and decanted repetitively with oxygenated buffer B containing 2.7 mM KCl, 137 mM NaCl, 1.5 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 1.5 mM EDTA, 1 mM dithiothreitol, and 10 mM glucose yielding enriched villus and crypt cells. The purity of the cell populations was determined by alkaline phosphatase (18) and sucrase activities. Cell viability was evaluated by the exclusion of 0.5% trypan blue. Only cell fractions with at least 90% dye exclusion after washing twice in KRB were used in these experiments. Cells were resuspended in 2 ml of KRB containing [1-^{14}C]acetate (1 μCi/ml). The protein concentration of the resuspended cells was adjusted to 5–10 mg/ml, and the cell fractions were incubated for 30 min at 37°C in a metabolic shaker. The reaction was interrupted by the addition of 6 ml of ethanolic KOH. After saponification, cholesterol was extracted, isolated by thin-layer chromatography, and counted as described above.

Incorporation of oleic acid into triglyceride and cholesterol and the absorption of glucose and leucine in everted intestinal rings

Small intestine was removed from rats maintained on either the fiber-free diet or the fiber-free diet to which

either 10% cellulose or 5% pectin was added. After the proximal half of the small intestine was flushed with cold 154 mM NaCl, the length:weight ratio was determined; a 1-g weight was attached to one end of the intestine, the length was measured, and the segment was weighed. Everted rings (10–20 mg) were prepared from this jejunal segment.

Rings were used to determine the incorporation of oleic acid into triglyceride and lecithin and the absorption of glucose and leucine. Some rings were weighed and placed in 25-ml flasks containing 2 ml of Ca^{2+}- and Mg^{2+}-free KRB saturated with 95% O_2:5%CO_2 (pH 7.4) (37°C), 10 mM sodium taurocholate, 1 mM oleic acid, and a trace amount of [1-^{14}C]oleic acid (0.2 μCi/μmol). Incubation solutions were prepared by sonication. After a 30-min incubation at 37°C in a metabolic shaker (oscillation 120/min), rings were removed, rinsed in ice-cold nonradioactive buffer, blotted, and homogenized in 0.5 ml of acid saline, pH 1. The solutions were oxygenated throughout the incubation period. Lipids were extracted according to the method of Folch, Lees, and Sloane Stanley (19). Triglyceride and phospholipid classes were isolated using thin-layer chromatography on activated silica gel G plates developed with either petroleum ether–ethyl ether–acetic acid 90:10:1 or chloroform–methanol–acetic acid–water 25:15:4:2. Areas corresponding to triglyceride or lecithin standards were visualized with iodine vapor and scraped into scintillation vials. Scintillation fluid was added to vials and the radioactivity was determined with a Beckman-LS-355 spectrometer using an external standard technique to correct for quenching. Rates of incorporation of oleic acid into both triglyceride and lecithin are expressed as nanomoles of oleic acid incorporated per min per g wet tissue weight (nmol/min per g wet wt).

Absorption of glucose and leucine was evaluated in jejunal rings incubated in flasks containing 2 ml of KRB saturated with 95% O_2:5% CO_2, [^{3}H]inulin (0.02 μCi/μmol) with either 10 mM D-[^{14}C-U]glucose (0.02 μCi/μmol) or 1 mM [^{14}C-U]leucine (0.02 μCi/μmol). After a 10-min incubation at 37°C in a metabolic shaker, each ring was removed, rinsed in cold buffer, blotted, transferred to a previously tared counting vial, and dried overnight at 110°C. After determining the dry tissue weight, 1 ml of tissue solubilizer was added and each vial was heated overnight at 50°C. After each vial had cooled to room temperature, scintillation fluid was added. Radioactivity was measured using an external standard technique to correct for quenching. Flux rates are expressed as nanomoles of probe molecule absorbed per min per 100 mg dry tissue weight (nmol/min per 100 mg). Flux was normalized to 1 millimolar concentration after accounting for the mass of the adherent mucosal fluid volume measured in microliters of solution per 100 mg dry tissue weight.

Mucosal RNA and DNA concentrations and enzyme activities

Mucosal scrapings were obtained from the distal half of the jejunum and homogenized in 14 vol of 0.28 M mannitol and 0.01 M Na_2HPO_4 (pH 7.4). Aliquots were obtained for (uronic acid content) pectin (20), RNA (21), DNA (22), protein (23), and disaccharidase activity (lactase, maltase, and sucrase (24) determinations and for microsome preparation. RNA was assayed using yeast RNA as a standard; DNA was assayed using calf thymus DNA as a standard. Acyl-CoA:1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase (E.C. 2.3.1.20) (lysolecithin acyltransferase) and cytidine diphosphocholine:1,2 diacyl-glycerocholine phosphotransferase (E.C. 2.7.8.2) (choline phosphotransferase) activities were determined in the microsomal fraction (25). Protein was determined on homogenates and microsomes using bovine serum albumin as a standard.

One ring from each animal was fixed in Bouin's solution. Sections for histological analysis were cut from the ring and stained with hematoxylin and eosin. Specimens were coded; the treatment group was unknown to the reviewer.

Statistical analysis

Data are expressed as means ± SEM. Data were analyzed using Student's unpaired *t*-test or a one-way analysis of variance where appropriate. When the analysis of variance was used, treatment variability was decomposed into two orthogonal comparisons: the first comparison involved animals receiving the fiber-free diet compared to those receiving cellulose or pectin supplementation; the second comparison involved animals receiving cellulose compared to rats receiving pectin.

RESULTS

Cholesterol synthesis in everted intestinal sacs is depicted in **Table 1.** Ileal cholesterol synthesis was more active than synthesis in the jejunum. Supplementation with pectin reduced jejunal and ileal cholesterol synthesis; pectin suppressed the ileal synthetic rate more than it suppressed the jejunal rate. Dietary supplementation with 0.7% cholesterol reduced ileal cholesterol synthesis when compared to synthesis in rats maintained on the fiber-free diet; cholesterol feeding did not affect jejunal cholesterol synthesis. The addition of pectin to the cholesterol-supplemented diet produced an additional decrement in jejunal and ileal cholesterol synthesis when compared to the synthesis in rats fed choles-

TABLE 1. Effect of diets on [1-^{14}C]acetate incorporation into cholesterol in everted intestinal sacs

Diet	n	Jejunum	Ileum
Fiber-free	6	311 ± 29	1685 ± 350
+10% Pectin	6	209 ± 6*	800 ± 84**
+0.7% Cholesterol	6	266 ± 54	425 ± 134*
+10% Pectin + 0.7% cholesterol	7	176 ± 37**	228 ± 24***
+2.5% Cholestyramine	4	448 ± 219	2769 ± 333

Incorporation is expressed as means (dpm/30 min per mg protein) ± SEM. n represents the number of animals maintained on each diet. *, $P < 0.005$; **, $P < 0.025$; ***, $P < 0.001$.

terol without fiber, and reduced cholesterol synthesis below those levels seen in animals maintained on either the fiber-free or pectin-supplemented diets. Cholestyramine ingestion stimulated cholesterol synthesis by 44% in the jejunum and 64% in the ileum.

There was less cholesterol synthesis observed in isolated crypt cells than in isolated villus cells (**Fig. 1**). Pectin ingestion reduced cholesterol synthesis in ileal crypt cells but did not affect the rate of cholesterol synthesis in either jejunal or ileal villus cells or in jejunal crypt cells.

Incorporation of oleic acid into triglyceride in jejunal rings was unaffected by the dietary status of the rats, whereas oleic acid incorporation into lecithin was affected by fiber ingestion. Incorporation of oleic acid into lecithin was reduced by one-third in animals maintained on either pectin or cellulose (**Table 2**).

Triglyceride synthesis occurs by both the monoglyceride and phosphatidic acid pathway; phospholipid synthesis occurs only by the phosphatidic acid pathway (26). The reduction in the rate of oleate incorporation into lecithin could be explained by a lack of sufficient substrate to maintain both the monoglyceride and phosphatidic acid pathways. Since phospholipid synthesis is stimulated by glucose (27) and depends upon the amount of α-glycerophosphate present, we incubated jejunal rings in the presence of glucose (5 mM) to increase α-glycerophosphate levels and again studied oleic acid incorporation into lecithin. The phosphatidic acid pathway was stimulated in rats maintained on both the fiber-free and pectin diets. In the presence of glucose, the rate of oleic acid incorporation into lecithin increased 80% (25.2 ± 4.8 to 45.0 ± 8.3 (SEM), n = 8 determinations) in rats ingesting the fiber-free diet and 40% (17.4 ± 3.0 to 24.4 ± 4.5, n = 8) in animals maintained on pectin. These increases were not statistically significant.

Jejunal uptake of both glucose and leucine was reduced in animals maintained on fiber when compared to rats maintained on a fiber-free diet (Table 2).

There was no difference in the specific activities of lysolecithin acyltransferase (located predominantly in villus cells) and choline phosphotransferase (located predominantly in crypt cells) among the three feeding groups (Table 2). Blood cholesterol concentration, tissue histology, length:weight ratio, RNA:DNA ratio, and mucosal uronic acid content were not affected by fiber ingestion (data not shown). Lactase, maltase, and sucrase activities were similar in rats fed the fiber-free diet and animals maintained on the cellulose diet. The activities of all three disaccharidases were increased in pectin-fed rats when compared to animals maintained on either the fiber-free and cellulose-supplemented diets (Table 2).

DISCUSSION

The objective of this study was to determine the effect of fiber ingestion on cholesterol and phospholipid synthesis. The data from intestinal rings, everted intestinal sacs, and isolated epithelial cell experiments support the hypothesis that prolonged ingestion of dietary fiber modifies intestinal cellular function and lipid metabolism.

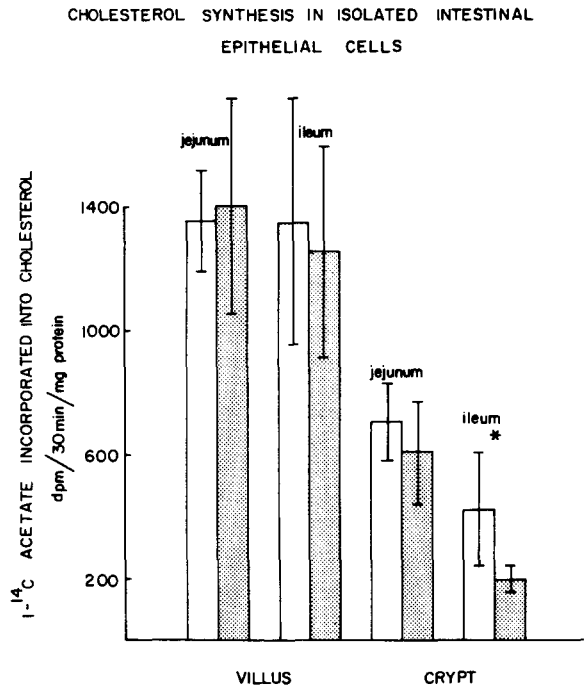

Fig. 1. Epithelial cell cholesterol synthesis [1-^{14}C]acetate incorporation. Epithelial cells from animals fed a fiber-free diet (open bars) or a 10% pectin-supplemented diet (stippled bars) were incubated in 2 ml of Krebs-Ringer bicarbonate buffer containing 1 μCi/ml [1-^{14}C]acetate and 5 mM glucose for 30 min. After saponification of the vial contents, cholesterol was extracted and chromatographed, and radioactivity was determined (see Methods). *, $P < 0.05$.

TABLE 2. Effect of fiber ingestion on oleic acid incorporation[a] into lecithin and triglyceride, on the absorption[b] of glucose and leucine, and on enzyme activities[c] in rat jejunum

	Lecithin	Triglyceride	Glucose	Leucine	Lactase	Maltase	Sucrase	Lysolecithin Acetyltransferase	Choline Phosphotransferase
+10% Cellulose (A)	13.4 ± 1.2 (7)[d]	643 ± 64 (7)	506 ± 59 (7)	32.9 ± 4.5 (6)	24.3 ± 1.0 (11)	202.0 ± 7.9 (11)	58.0 ± 5.4 (11)	20.8 ± 0.9 (11)	2.5 ± 0.8 (11)
+5% Pectin (B)	13.8 ± 1.1 (8)	760 ± 82 (8)	408 ± 55 (8)	26.9 ± 2.7 (7)	30.7 ± 1.7 (11)	253.5 ± 10.2 (11)	81.4 ± 6.3 (11)	21.3 ± 1.6 (11)	2.3 ± 0.4 (11)
A vs. B	NS	NS	NS	NS	*	*	***	NS	NS
Fiber-free diet (C)	20.8 ± 1.6 (15)	731 ± 66 (15)	611 ± 38 (14)	46.1 ± 5.5 (13)	25.8 ± 1.1 (18)	198.0 ± 8.0 (18)	58.0 ± 3.6 (18)	21.4 ± 1.2 (18)	2.6 ± 0.8 (18)
C vs. A + B	**	NS	**	**	NS	**	*	NS	NS

[a] Incorporation is expressed as means (nmol/30 min per g wet wt) ± SEM (n).
[b] Absorption is expressed as means (nmol/min per 100 ml dry tissue wt) ± SEM (n).
[c] Activity is expressed as means (μmol/min per mg protein) ± SEM (n).
[d] (n) represents the number of animals maintained on each diet.
*, $P < 0.025$; **, $P < 0.005$; ***, $P < 0.001$; NS, not significant.

In addition, our in vitro studies: *1*) demonstrate diminished glucose and amino acid transport in intestinal rings from rats fed fiber-supplemented diets; *2*) confirm in vivo studies that demonstrated reduced glucose absorption in the jejunum of rats maintained on diets supplemented with either 10% cellulose or 5% pectin (15); and *3*) support the concept that fiber ingestion alters the epithelial cell luminal surface (14).

Retained fiber could conceivably increase the unstirred water layer, and inhibit intestinal uptake of solutes without altering the intestinal membrane (13). (We did not detect pectin in the jejunum after an 18-hr fast using the carbazole method and assume this also precludes the presence of cellulose.) Retention of fiber resulting in a thickened, jejunal unstirred water layer, therefore, cannot explain the diminished glucose or leucine uptake observed in these experiments. The in vitro studies exclude any possibility that hormonal or vascular alterations could be responsible for our previously reported in vivo data (15). Our data suggest that sustained fiber ingestion results in alterations of the intestinal membrane; the changes in disaccharidase activity after prolonged pectin ingestion support this conclusion.

Pectin ingestion is believed to induce hypocholesterolemia in man (10, 11) and rats (28) by increasing neutral and bile acid sterol fecal excretion. In women ingesting pectin, Stasse-Wolthuis et al. (29) observed reductions in serum cholesterol levels without concomitant changes in neutral or acidic sterol excretion. This experiment (29) substantiated observations made in rats that fecal sterols did not increase with pectin feeding (30, 31). These studies (29–31) suggest that serum cholesterol levels might be influenced by events unrelated to bile acid metabolism.

In our study intestinal cholesterol synthesis was suppressed by pectin feeding while blood cholesterol levels remained unchanged. Reiser et al. (32) noted that pectin feeding increased hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (E.C. 1.1.1.34) activity even though serum cholesterol levels were unaltered. We did not measure hepatic or intestinal HMG-CoA reductase activity but our data suggest that pectin feeding may have different effects on intestinal and hepatic MHG-CoA reductase activity.

Small intestinal cholesterol synthesis is regulated by flux of bile acids through the mucosa. Dietschy and Siperstein (33) described a lower synthetic rate in the jejunum relative to the ileum and Dietschy (34) emphasized the profound stimulatory effect that diversion of bile acids had on intestinal cholesterogenesis, especially synthesis in the ileum. The circadian rhythm of intestinal cholesterol synthesis, while not as marked as that of hepatic cholesterol synthesis, is concordant with HMG-CoA reductase activity in intestinal epithelial cells (35–37). The role of cholesterol in suppressing intes-

tinal cholesterol synthesis is unclear. In one study cholesterol supplementation suppressed intestinal cholesterol synthesis (38), while in another study dietary cholesterol did not suppress intestinal cholesterogenesis (39). Cholesterol supplementation did suppress intestinal HMG-CoA reductase activity (40).

Our studies demonstrate that the fiber content of the diet affects sterol synthesis and suggest that crypt cells are responsive to these dietary manipulations. Cholesterol synthesis in intestinal villus cells is apparently more active than cholesterol synthesis in crypt cells (Fig. 1). These findings are consistent with those of Merchant and Heller (37) who found that isolated villus cells incorporated up to 2-fold more acetate into digitonin-precipitable sterol than did crypt cells, but are not consistent with those of Shakir et al. (41) who observed that acetate incorporation into lipids was 5-fold greater in isolated crypt cells than in upper villus cells with cholesterol synthesis accounting for one-quarter of the total lipid synthesized.

If the alterations in cholesterol metabolism observed with pectin ingestion are related to intraluminal bile acid binding and a subsequent increase in bile acid loss, intestinal cholesterol synthesis should increase with pectin supplementation (42). In our study, pectin ingestion resulted in a reduction in ileal and jejunal cholesterol synthesis. (Only the ingestion of cholestyramine, a bile acid-binding resin, enhanced intestinal cholesterol synthesis in our study.) Our data suggest that pectin ingestion alters lipid metabolism by a mechanism other than that of increasing intestinal bile acid loss.

The ingestion of both pectin, a water-soluble dietary fiber, and cellulose, an insoluble fiber, diminished the incorporation of oleic acid into lecithin while leaving the incorporation of oleate into triglyceride unaffected. Since glucose provides α-glycerophosphate for the phosphatidic acid pathway (27), the addition of 5 mM glucose during incubation ensured that a sufficient amount of α-glycerophosphate was present for phospholipid synthesis and that lack of substrate was not responsible for the difference in lecithin synthesis noted between dietary groups.

Our studies suggest that intestinal cholesterol and phospholipid synthesis is suppressed by long-term pectin administration. The significance of these findings would be enhanced if alterations in intestinal chylomicron and/or lipoprotein secretion could be demonstrated as a result of dietary pectin supplementation.

Manuscript received 19 February 1982, in revised form 12 October, 1982, and in re-revised form 31 January 1983.

REFERENCES

1. Schwartz, S. E., C. M. Starr, P. G. Holtzapple. 1980. Effects of dietary fiber (F) on carbohydrate and amino acid absorption and on triglyceride (T) and phospholipid metabolism in rat intestine. *Gastroenterology.* **78:** 1256.
2. Holtzapple, P. G., S. E. Schwartz, C. M. Starr, and S. Bachman. 1981. Effect of chronic pectin ingestion on intestinal cholesterol metabolism in rats. *Gastroenterology.* **80:** 1179.
3. Schwartz, S. E., P. G. Holtzapple, C. M. Starr, and S. Bachman. 1981. Effect of chronic pectin ingestion on intestinal cholesterol synthesis in rats. *Clin. Res.* **29:** 714A.
4. Mathe, D., C. Lutton, J. Rautureau, T. Coste, E. Gouffier, J. C. Sulpice, and F. Chevallier. 1977. Effects of dietary fiber and salt mixtures on the cholesterol metabolism of rats. *J. Nutr.* **107:** 466–474.
5. Tsai, A. C., J. Elias, J. J. Kelley, R. C. Lin, and J. R. K. Robson. 1976. Influence of certain dietary fibers on serum and tissue cholesterol levels in rats. *J. Nutr.* **106:** 118–123.
6. Wells, A. F., and B. H. Ershoff. 1961. Beneficial effects of pectin in prevention of hypercholesterolemia and increase in liver cholesterol on cholesterol-fed rats. *J. Nutr.* **74:** 87–92.
7. Chen, W. L., and J. W. Anderson. 1979. Effects of plant fiber in decreasing plasma total cholesterol and increasing high-density lipoprotein cholesterol. *Proc. Soc. Exp. Biol. Med.* **162:** 310–313.
8. Jenkins, D. J. A., A. R. Leeds, M. A. Gassull, H. Houston, D. V. Goff, and M. Hill. 1976. The cholesterol-lowering properties of guar and pectin. *Clin. Sci. Molec. Med.* **51:** 8p–9p.
9. Anderson, J. W., and W. L. Chen. 1979. Plant fiber: carbohydrate and lipid metabolism. *Am. J. Clin. Nutr.* **32:** 346–363.
10. Kay, R. M., and A. W. Truswell. 1977. Effects of citrus pectin on blood lipids and fecal steroid excretion in man. *Am. J. Clin. Nutr.* **30:** 171–175.
11. Miettinen. T. A., and S. Tarpila. 1977. Effect of pectin on serum cholesterol, fecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals. *Clin. Chim. Acta.* **79:** 471–477.
12. Jenkins, D. J. A., T. M. S. Wolever, A. R. Leeds, M. A. Gassull, P. Haisman, J. Dilawari, D. V. Goff, G. L. Metz, and K. G. M. M. Alberti. 1978. Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br. Med. J.* **1:** 1392–1394.
13. Johnson I. T., and J. M. Gee. 1981. Effect of gel-forming gums on the intestinal unstirred layer and sugar transport in vitro. *Gut.* **22:** 398–403.
14. Cassidy, M. M., F. G. Lightfoot, L. E. Grau, J. A. Story, D. Kritchevsky, and G. V. Vahouny. 1981. Effect of chronic intake of dietary fibers on the ultrastructural topography of rat jejunum and colon. A scanning electron microscopy study. *Am. J. Clin. Nutr.* **34:** 218–228.
15. Schwartz, S. E., and G. D. Levine. 1980. Effects of dietary fiber on intestinal glucose absorption and glucose tolerance in rats. *Gastroenterology.* **79:** 833–836.
16. Schwartz, S. E., G. D. Levine, and C. M. Starr. 1982. Effect of dietary fiber on intestinal ion fluxes in rats. *Am. J. Clin. Nutr.* **36:** 1102–1105.
17. Weiser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. *J. Biol. Chem.* **248:** 2536–2541.
18. Panini, S. R., G. Lehrer, D. M. Rogers, and H. Rudney. 1979. Distribution of 3-hydroxy-3-methylglutaryl coenzyme A reductase and alkaline phosphatase activities in isolated ileal epithelial cells of fed, fasted, cholestyramine-

fed, and 4-aminopyrazolo[3,4-*d*]pyrimidine-treated rats. *J. Lipid Res.* **20:** 879–889.
19. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
20. Bitter, T., and H. M. Muir. 1962. A modified uronic acid carbazole reaction. *Anal. Biochem.* **4:** 330–334.
21. Almog, R., and T. L. Shively. 1978. A modified original test for the specific determination of RNA. *Anal. Biochem.* **91:** 130–137.
22. Leyva, A., and W. Kelley. 1974. Measurements of DNA in cultured human cells. *Anal. Biochem.* **62:** 173–179.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
24. Dahlquist, A. 1968. Assay of intestinal disaccharidases. *Anal. Biochem.* **22:** 99–107.
25. Holtzapple, P. G., C. M. Starr, and T. Morck. 1980. Phosphatidylcholine synthesis in the developing small intestine. *Biochem. J.* **186:** 399–403.
26. Shiau, Y. F., and P. G. Holtzapple. 1980. Effect of insulin on in vitro fatty acid esterification of rat jejunum. *Am. J. Physiol.* **238:** E364–E370.
27. Shiau, Y. F., W. B. Long, and J. B. Weiss. 1978. Effect of sugar and monogylceride on fatty acid esterification. *Am. J. Physiol.* **234:** E236–E242.
28. Balmer, J., and D. B. Zilversmit. 1974. Effects of dietary roughage on cholesterol absorption, cholesterol turnover and steroid excretion in the rat. *J. Nutr.* **104:** 1319–1328.
29. Stasse-Wolthuis, M., H. F. F. Albers, J. G. C. Van Jerveren, I. W. deJong, J. G. A. J. Hautvast, R. J. J. Hermus, M. B. Katan, W. G. Brydon, and M. A. Eastwood. 1980. Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids, and colon function. *Am. J. Clin. Nutr.* **33:** 1745–1756.
30. Leveille, G. A., and H. E. Sauberlich. 1966. Mechanism of the cholesterol-depressing effect of pectin in the cholesterol-fed rat. *J. Nutr.* **88:** 209–214.
31. Nomani, M. Z. A., C. J. Bradac, H. L. Lai, and A. L. Watne. 1979. Effect of dietary fiber fractions on the fecal output and steroids in the rat. *Nutr. Rep. Int.* **20:** 269–279.
32. Reiser, B., G. R. Henderson, B. C. O'Brien, and J. Thomas. 1977. Hepatic 3-hydroxy-3-methylglutoryl coenzyme A reductase of rats fed semipurified and stock diets. *J. Nutr.* **107:** 453–457.
33. Dietschy, J. M., and M. D. Siperstein. 1965. Cholesterol synthesis by the gastrointestinal tract: localization and mechanisms of control. *J. Clin. Invest.* **44:** 1311–1327.
34. Dietschy, J. M. 1968. The role of bile salts in controlling the rate of intestinal cholesterogenesis. *J. Clin. Invest.* **47:** 286–300.
35. Edwards, P. A., H. Muroya, and R. G. Gould. 1972. In vivo demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. *J. Lipid Res.* **13:** 396–401.
36. Muroya, H., H. S. Sodhi, and R. G. Gould. 1977. Sterol synthesis in intestinal villi and crypt cells of rats and guinea pigs. *J. Lipid Res.* **18:** 301–308.
37. Merchant, J. L., and R. A. Heller. 1977. 3-Hydroxy-3-methylglutaryl coenzyme A reductase in isolated villous and crypt cells of the rat ileum. *J. Lipid Res.* **18:** 722–733.
38. Ho, K. J. 1975. Effect of cholesterol feeding on circadian rhythm of hepatic and intestinal cholesterol biosynthesis in hamsters. *Proc. Soc. Exp. Biol. Med.* **150:** 271–277.
39. Dietschy, J. M., and W. G. Gamal. 1971. Cholesterol synthesis in the intestine of man: regional differences and control mechanism. *J. Clin. Invest.* **50:** 872–880.
40. Bochenek. W. J., and J. B. Rodgers. 1979. Dietary regulation of 3-hydroxy-3-methylglutaryl CoA reductase from rat intestine. *Biochim. Biophys. Acta.* **575:** 57–62.
41. Shakir, K. M. M., S. G. Sundaram, and S. Margolis. 1978. Lipid synthesis in isolated intestinal cells. *J. Lipid Res.* **19:** 433–442.
42. Ebihara, K., S. Kiriyama, and M. Manabe. 1979. Cholesterol-lowering activity of various natural pectins and synthetic pectin-derivatives with different physico-chemical properties. *Nutr. Rep. Int.* **20:** 519–526.