Effects of Chitosan Hydrolysates on Lipid Absorption and on Serum and Liver Lipid Concentration in Rats

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The enzymatic hydrolysates of chitosan with average molecular weights range from 5000 to 20 000 and with low viscosity interfered with intestinal absorption of cholesterol in lymph fistulated rats and increased fecal excretion of neutral steroids in rats fed a cholesterol-enriched diet. The hydrolysates exerted a cholesterol-lowering activity, and it was more marked in the liver than in the serum. They also reduced liver triglyceride significantly. Chitosan hydrolysates with average molecular weights of 10 000–20 000 were as effective as high-viscous chitosan with a molecular weight of 50 000. The results indicate usability of low-viscosity chitosan hydrolysate as a hypocholesterolemic agent.

Chitosan, a polymer of glucosamine, exerts a marked hypocholesterolemic activity and also decreases hepatic cholesterol and triglyceride in experimental animals (Sugano et al., 1978, 1980, 1988; Kobayashi et al., 1979; Nagyvary et al., 1979; Jennings et al., 1988; Hirano et al., 1990; Ebihara and Schneeman, 1990) accompanying an interference with lymphatic absorption of cholesterol and fatty acid (Vahouny et al., 1983; Ikeda et al., 1990) and an increase of fecal excretion of neutral steroids (Sugano et al., 1978, 1980). The recommendation of chitosan as a hypocholesterolemic agent is reasonable, since its side effect is lower as compared with that of cholestyramine, an anion-exchange resin having a strong bile acid binding capacity (Gordon and Besch-Williford, 1984; Jennings et al., 1988).

However, the high viscosity of chitosan in solution limits its use as a food additive as in the case of guar gum, and it is desirable to prepare chitosans with low viscosity without largely influencing its desirable functions such as hypocholesterolemic potential. Our previous study showed that various commercially available chitosans with viscosity between 17 and 1620 cP as 1% solution have an equal hypocholesterolemic activity when fed to rats (Sugano et al., 1988). In contrast, glucosamine oligomer composed mainly of three to five amino sugar residues did not show an activity (Sugano et al., 1988). Thus, some molecular size appears to be required for exerting a desirable effect. In a preliminary study in which the effect of low molecular weight chitosans on serum and liver cholesterol levels was examined, we found that the partial hydrolysate with a molecular weight of approximately 10 000 still maintains a significant hypocholesterolemic activity (Sugano et al., 1992). The present study focused on the effect of chitosan hydrolysates with varying molecular weights on lipid metabolism and lymphatic lipid absorption in rats.

MATERIALS AND METHODS

Preparation of Enzymatic Hydrolysates of Chitosan. Chitosan hydrolysates were prepared from a relatively low molecular weight chitosan (average molecular weight 50 000, Kimitsu Chemical Industries Co., Tokyo) by chitosanase [EC 3.2.1.99] from *Verticillium* sp. AF9-V-156. The bacterium was cultured in the medium containing chitosan, and the concentrate of the ultrafiltrate to the broth was used as an enzyme source. Five percent chitosan dissolved in 3% lactic acid was hydrolyzed at 30 °C for 18 h by adding varying amounts of the chitosanase to yield the hydrolysates with different molecular weights. The hydrolysates were isolated either by the alkaline precipitation method (LP-5, LP-10, and LP-20) or by the ion-exchange (Dowex MSC-1, Muromachi Kagaku Co., Tokyo) method (LP-2) depending on the molecular size, and lyophilized. The molecular weight distribution of the products was measured by gel filtration on a Sephacryl S-200 column (Pharmacia LKB Biotechnology, Tokyo). Dextran (MW 510 000, 71 000, 39 000, and 9400, Sigma Chemical Co., St. Louis, MO), chitohexaose hydrochloride (MW 1200, Seikagaku Corp., Tokyo), and glucosamine hydrochloride (MW 216, Wako Pure Chemicals, Osaka) were used as molecular weight markers. The degree of deacetylation of the preparations was measured by the colloidal titration method (Kina et al., 1974). Viscosity of these hydrolysates was measured at 25 °C as 1% solution dissolved in 0.5% acetic acid by using an E-type viscometer (Tokyo Keiki Co., Tochigi, Japan). The bile acid binding capacity in vitro of the preparations was measured as described elsewhere by incubating 20 mg of the preparations with 2 μmol of sodium taurocholate and 5×10^{-4} μmol of sodium [^{14}C-*carboxy*]taurocholate (56 mCi/mmol, Amersham, Buckinghamshire, England) at 37 °C for 2 h (Sugano et al., 1990).

Animal Feeding Study. Male Sprague-Dawley rats, weighing on average 88 g, were fed experimental diets ad libitum for 14 days. The control diet according to the recommendation of the American Institute of Nutrition (1977) contained (weight percent casein (20), lard (10), vitamin mixture (1), mineral mixture (3.5), choline bitartrate (0.2), DL-methionine (0.3), cholesterol (0.25), sodium cholate (0.06), cellulose (4), corn starch (15), and sucrose (to 100). Testing materials were added to the diet at the 2% level at the expense of cellulose. Blood was withdrawn from the tail vein 7 days after feeding experimental diets, and serum cholesterol was measured enzymatically (Cholesterol C-Test, Wako Pure Chemicals).

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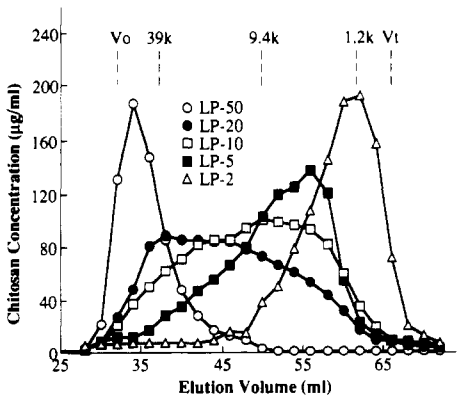

Figure 1. Gel filtration pattern of the hydrolysates of chitosan. Numbers at the top represent molecular weights of the markers. The average molecular weights of LP-50, LP-20, LP-10, LP-5, and LP-2 were 50 000, 20 000, 10 000, 5000, and 2000, respectively.

The rats were killed by decapitation at 1 p.m. after fasting for 7 h. Feces were collected for 2 days between days 11 and 13.

Lymphatic Absorption of Cholesterol and Fatty Acids. Male Sprague-Dawley rats weighing 260–300 g were surgically fitted with a left thoracic lymphatic cannula and an indwelling catheter in the stomach (Ikeda et al., 1990). They received a fresh prepared test emulsion (3 mL per rat) via stomach tube, and the lymph was collected periodically for 24 h in a tube containing ethylenediaminetetraacetic acid. The emulsion contained (in 3 mL of water) taurocholate (200 mg) (Nacalai Tesque, Kyoto), albumin (50 mg) (fatty acid-free, Miles Inc., Kankakee, IL), cholesterol (10 mg), [^{14}C]cholesterol (1 μCi) (60 mCi/mmol, NEN Research Products, Boston, MA), triolein (200 mg) (Sigma Chemical Co.), and test material (50 mg). Control lymph was collected for 2 h prior to the administration of the emulsion.

Lipid Analyses. Serum and liver lipids were analyzed for cholesterol, triglyceride, and phospholipid as reported elsewhere (Sugano et al., 1990). An aliquot of the lymph was transferred into a scintillation vial, and 10 mL of Aquasol II (NEN Research Products) was added. The radioactivity was counted by liquid scintillation counter (LSC-900, Aloka Co., Tokyo). The lymph lipid was extracted, and the fatty acid composition was analyzed by gas–liquid chromatography (GLC) using pentadecanoic acid (Aldrich Chemical Co., Milwaukee, WI) as an internal calibration standard (Ikeda et al., 1991). The fatty acid absorption rate was calculated by subtracting the fatty acid amount in the control lymph from that of the groups administered a test emulsion. Fecal steroids were analyzed by GLC using 5α-cholestane (Nacalai Tesque) and 23-nordeoxycholic acid (Steraloids Inc., Winston, NH) as internal standards for neutral and acidic steroids, respectively (Tanaka et al., 1984; Sugano et al., 1984).

RESULTS

Molecular weight distributions of the hydrolysates are shown in Figure 1. Average molecular weights of these preparations were 2000 (LP-2), 5000 (LP-5), 10 000 (LP-10, and 20 000 (LP-20) as compared with 50 000 of the unhydrolyzed chitosan (LP-50). The extent of deacetylation was approximately 80% in all preparations. Therefore, the effect of molecular weight can be compared as a sole variable. Table I shows some properties of chitosan hydrolysates used in this study. The viscosity of the hydrolysates was lower than that of chitosan having molecular weight of 50 000 and was almost equivalent to that of water at the measured condition. The effect of pH on the solubility of chitosan preparations was also measured as shown in Table I. LP-50 precipitated at pH 6.6, whereas LP-5, LP-10, and LP-20 formed precipitate at pH 7.0–8.3. LP-2 did not form precipitate up to pH 10. The bile acid binding capacity in vitro was comparable among the preparations, and it was higher than that of chitin but lower than that of cholestyramine.

Table I. Characterization of Chitosan Hydrolysates

preparation	av mol wt	viscosity,[a] cP	pH precipitated	bile acid binding capacity,[b] nmol/20 mg
LP-2	2000	1.1	c	0.50
LP-5	5000	1.3	7.8–8.3	0.48
LP-10	10000	1.6	7.0–7.4	0.52
LP-20	20000	1.9	7.1–7.3	0.55
LP-50	50000	11.0	6.6	0.52

[a] Viscosity was measured as 1% solution in 0.5% acetic acid (average of two determinations). [b] The binding capacities of chitin and cholestyramine were 0.15 and 1.46, respectively (average of two determinations). [c] No precipitate formed up to pH 10.

Table II. Effects of Different Chitosan Hydrolysates on Growth Parameters and Liver Weight[a]

group	body wt, g initial	body wt, g gain	food intake, g/day	rel liver wt, g/100 g
cellulose	88 ± 3	111 ± 7[a]	17.9 ± 0.8[a]	6.55 ± 0.42[a]
LP-2	88 ± 3	77 ± 8[b]	13.6 ± 0.7[b]	4.82 ± 0.17[b]
LP-5	88 ± 3	105 ± 2[a]	17.7 ± 0.4[a]	4.98 ± 0.15[bc]
LP-10	88 ± 3	97 ± 6[a]	16.9 ± 0.5[a]	5.00 ± 0.13[bc]
LP-20	88 ± 3	108 ± 8[a]	17.7 ± 0.8[a]	5.50 ± 0.22[c]
LP-50	88 ± 3	107 ± 5[a]	17.8 ± 0.4[a]	5.07 ± 0.16[bc]

[a] Mean ± SE of six rats. Values not sharing a common letter are significantly different at $p < 0.05$.

As shown in Table II, food intake and growth of rats fed chitosan preparations were comparable with those of rats fed cellulose except for the LP-2 group, in which these parameters were unexpectedly low. The reduction of food intake and, hence, weight gain in the LP-2 group was observed during the second week for unknown reason. Enlargement of the liver due to ingestion of a cholesterol-enriched diet was significantly prevented in all groups of rats fed chitosan preparations.

The concentration of serum cholesterol at day 7 was significantly lower in rats fed LP-5, LP-10, LP-20, and LP-50 preparations than in those fed cellulose (Table III). The LP-2 preparation did not show a hypocholesterolemic effect at day 7 when the growth parameters were comparable with those of the other hydrolysate groups. However, after 14 days, the effect of the hydrolysates became not significant, although the level still tended to be lower in the hydrolysate groups as compared with the cellulose group. This was mainly due to an unpredictable reduction of serum cholesterol in the cellulose group at day 14. The LP-2 preparation again did not reduce serum cholesterol.

The concentration of liver cholesterol was significantly reduced by feeding chitosan hydrolysates with molecular weights above 5000 as compared with cellulose, whereas the hydrolysates with a molecular weight of 2000 did not show any effect (Table III). The preparations with molecular weights above 10 000 were more effective than those with molecular weights lower than these preparations. All of the hydrolysates reduced liver triglyceride to a comparable extent as compared with cellulose.

As shown in Figure 2, the weight of dried feces was significantly higher in the LP-20 and LP-50 groups than in the cellulose group, whereas it was significantly lower in the LP-2 group, presumably due to a reduction of the

Table III. Effects of Different Chitosan Hydrolysates on Serum and Liver Lipid Levels[a]

	serum lipids, mg/dL				liver lipids, mg/g		
	CHOL						
group	7 days	14 days	TG	PL	CHOL	TG	PL
cellulose	213 ± 21[a]	147 ± 8[ab]	149 ± 28	152 ± 4[ac]	34.2 ± 3.2[a]	109 ± 16[a]	28.0 ± 1.6[ac]
LP-2	187 ± 13[a]	162 ± 12[b]	109 ± 43	129 ± 18[b]	32.6 ± 2.6[a]	44.9 ± 7.0[b]	31.5 ± 0.6[b]
LP-5	147 ± 7[b]	127 ± 9[a]	110 ± 13	142 ± 9[ab]	26.1 ± 1.1[b]	55.5 ± 5.6[b]	30.8 ± 0.1[ab]
LP-10	132 ± 4[bc]	114 ± 9[a]	121 ± 11	161 ± 5[ac]	13.4 ± 1.7[c]	39.7 ± 4.5[b]	29.7 ± 0.3[abc]
LP-20	111 ± 6[c]	105 ± 8[a]	138 ± 11	172 ± 7[c]	14.2 ± 2.2[c]	50.4 ± 6.6[b]	27.9 ± 1.0[c]
LP-50	142 ± 7[bc]	116 ± 8[a]	133 ± 20	162 ± 9[ac]	16.1 ± 2.0[c]	50.9 ± 5.7[b]	29.7 ± 0.8[abc]

[a] Mean ± SE of six rats. CHOL, cholesterol; TG, triglyceride; PL, phospholipid. Values not sharing a common letter are significantly different at $p < 0.05$.

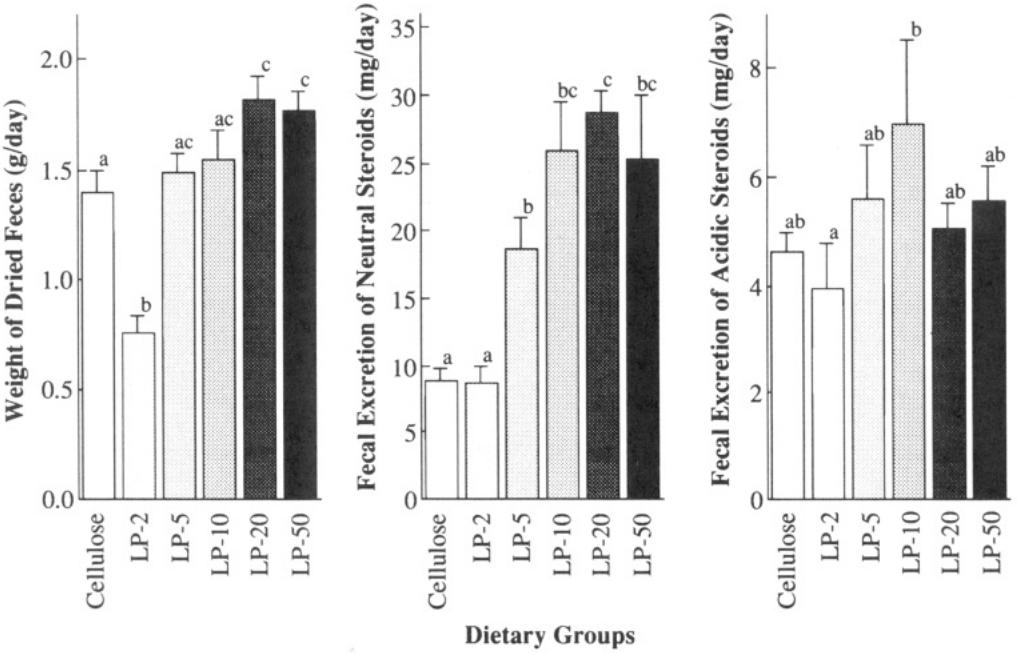

Figure 2. Effects of chitosan hydrolysates on fecal excretion of neutral and acidic steroids (mean ± SE of six rats). (a–c) Values not sharing a common letter are significantly different at $p < 0.05$.

amounts of diets consumed. All chitosan hydrolysates except for LP-2 significantly increased fecal excretion of neutral steroids as cholesterol and coprostanol. The hydrolysates with average molecular weights above 10 000 were more effective in enhancing fecal excretion of neutral steroids, whereas the effect of LP-5 was moderate, although the effect was still significant. The effect on the composition of neutral steroids was diverse depending on the preparations (data not shown). Excretion of total acidic steroids was slightly increased in rats fed chitosan hydrolysates except for LP-2. Although the difference was not significant, the excretion in the LP-10 group was 1.5-fold higher than in the cellulose group.

Lymphatic absorption of radioactive cholesterol is shown in Figure 3. The rats given cellulose most effectively absorbed cholesterol during 24 h. Cholesterol absorption in the LP-2 group was comparable with the cellulose group at 24 h after administration, but it was lower at 6 and 9 h. The lowering effects of LP-5, LP-20, and LP-50 on cholesterol absorption were comparable and were effective for 24 h. The effect of LP-10 was moderate until 9 h after administration, but at 24 h the absorption rate was reduced to a similar extent as in the effective preparations.

As shown in Figure 4, absorption of oleic acid administered as triolein was comparable among the groups at 24 h after administration, but there was a considerable difference in the absorption pattern. The rats given cellulose most effectively absorbed oleic acid at 3, 6, and 9 h after administration. Apparent delayed absorption of

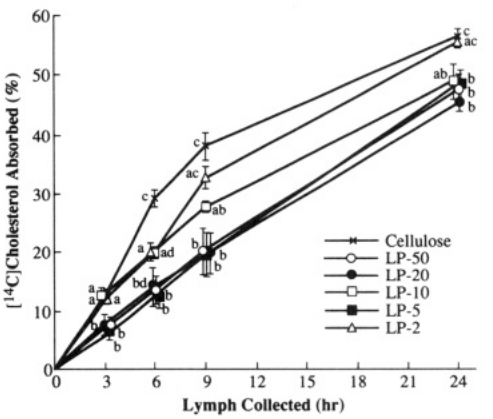

Figure 3. Effects of chitosan hydrolysates on lymphatic absorption of cholesterol in rats (mean ± SE of six rats). (a–d) Values not sharing a common letter are significantly different at $p < 0.05$.

oleic acid was observed in rats given LP-5, LP-20, and LP-50 until 9 h after administration. The interfering effect of LP-10 on the initial stage of oleic acid absorption was rather moderate.

DISCUSSION

Our results clearly showed that chitosan hydrolysates having average molecular weights of 10 000 and 20 000 effectively lowered plasma and liver cholesterol concentration, and the effect was comparable with the molecular

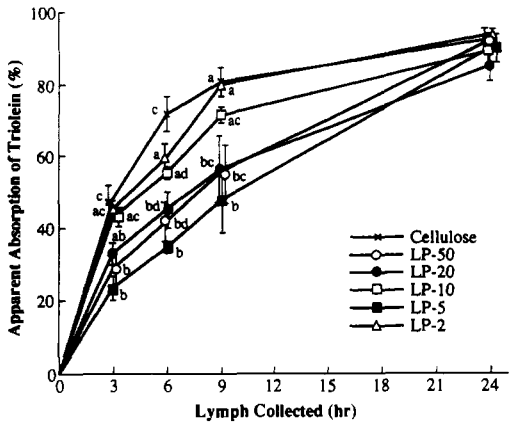

Figure 4. Effects of chitosan hydrolysates on lymphatic absorption of triglyceride (triolein) in rats (mean ± SE of six rats). (a–d) Values not sharing a common letter are significantly different at $p < 0.05$.

weight 50 000 chitosan. Chitosan hydrolysate of molecular weight 5000 showed moderate lowering effects. Results of fecal excretion of neutral steroids in the feeding study and of lymphatic absorption of cholesterol in lymph-cannulated rats indicated that the cholesterol-lowering activity of chitosan hydrolysates was ascribed to the inhibition of cholesterol absorption and, hence, the increase in fecal neutral steroids excretion.

The lymphatic absorption of oleic acid as triolein was delayed, and the concentration of liver triglyceride was decreased by chitosan hydrolysates as compared with cellulose. However, at 24 h after administration, the delaying effect disappeared. In contrast, viscous chitosans reduced not only absorption of both cholesterol and fatty acids (Vahouny et al., 1983; Ikeda et al., 1990) but also the concentration of liver triglyceride (Sugano et al., 1988), suggesting a possible preferable effect of high molecular weight preparations on this parameter.

Several mechanisms have been proposed for the inhibitory effect of chitosan on lipid absorption. It has been believed that the high viscosity may be primarily responsible for the inhibition of cholesterol and fatty acid absorption by chitosans as in the case of guar gum and pectin (Ikeda et al., 1990). However, since chitosan hydrolysates with low viscosity inhibited cholesterol absorption in this study and since chitosans with viscosity higher than 17 cP were equally hypocholesterolemic (Sugano et al., 1988), it is not obvious whether the mechanisms proposed for viscous fiber, the reduction of diffusion of micellar lipids to intestinal walls, and the coating effect on inner intestinal wall (Vahouny, 1982; Edwards, 1988; Furda, 1990) could be applicable to chitosan hydrolysates.

In addition, the property of chitosan as an anion exchanger may at least in part be responsible for its prominent hypocholesterolemic activity as presumed from the action of cholestyramine. Chitosan, as an anion-exchanger, binds bile acid and fatty acid by ionic bond at pH lower than 6.0 (Furda, 1983; Nauss et al., 1983), and hence, the binding may occur in the stomach and/or in the jejunal lumen. Consequently, chitosan may disturb micellar solubility of cholesterol and fatty acid and inhibit their absorption. However, the cholesterol-lowering activity was not equivalent among the hydrolysate preparations having different molecular weights, although the degree of deacetylation of the hydrolysates used in this study was the same for each preparation. Therefore, the influence of chitosan hydrolysates on lipid absorption as an anion exchanger is not necessarily unequivocal.

Furda (1983) proposed two mechanisms for luminal action of chitosan, "polar entrapment" and "disintegration" of mixed micelles. Precipitation and aggregation of chitosan at pH 6.0–6.5 has been thought to be concerned with the entrapment of whole micelles, and ionic bonding may be involved in disintegration. Our results showed that some molecular size may be required for the appearance of the lipid-lowering effect of chitosan hydrolysates and that chitosan hydrolysate having average molecular size of 2000, which did not precipitate at any pH, did not show any lipid-lowering effect. Therefore, entrapment of mixed micelles may be one possible mechanism for the effect of chitosan hydrolysates, although it seems likely that the cholesterol-lowering action of chitosan can be exerted through a combination of diverse mechanisms.

Acidic steroid excretion slightly increased when rats were fed chitosan hydrolysates with molecular weights above 5000, contrary to the previous observations with high molecular weight chitosans in which acidic steroid excretion did not increase (Sugano et al., 1980). Since chitosan can act as an anion exchanger, the increase in acidic steroid excretion by chitosan is reasonable (Ebihara and Schneeman, 1989), although the evidence is controversial. Since bile acid and fatty acid entrapped by chitosan in the stomach and/or in the upper intestine are released at pH > 6.0, these anions will become available for absorption in the middle to lower intestine, and hence, bile acid is normally reabsorbed in the lower intestine. Therefore, it can be assumed that enzymatic hydrolysis of chitosan influences the pH of the released entrapped bile acid from the hydrolysates and hence, a part of bile acid may be excreted to feces.

The delayed absorption of fatty acids observed in our lymph-cannulated rats can also be explained by their binding with chitosan hydrolysates at lower pH in the stomach and/or the upper intestinal lumen followed by the release at higher pH in the lower intestine. The slowed absorption of fatty acid may be concerned with the reduction of hepatic triglyceride by dietary chitosan (Sugano et al., 1980, 1988).

In conclusion, the chitosan hydrolysates with low viscosity were found to maintain the preferable function of viscous chitosan. These preparations, therefore, can be applied for various foods as a physiologically functional factor. However, the cholesterol-lowering action was dependent on the molecular weight of the hydrolysates, and a lower limit may exist.

LITERATURE CITED

American Institute of Nutrition. Report of the AIN ad hoc committee on standards for nutrition studies. *J. Nutr.* **1977**, *107*, 1340–1348.

Ebihara, K.; Schneeman, B. O. Interaction of bile acids, phospholipids, cholesterol and triglyceride with dietary fibers in the small intestine of rats. *J. Nutr.* **1989**, *119*, 1100–1106.

Edwards, C. A. Physiological effect of fiber. In *Dietary Fiber*; Kritchevsky, D., Bonfield, C., Anderson, J. W., Eds.; Plenum Press: New York, 1988; pp 167–178.

Furda, I. Aminopolysaccharides—their potential as dietary fiber. In *Unconventional sources of dietary fiber*; Furda, I., Ed.; American Chemical Society: Washington, DC, 1983; pp 105–122.

Furda, I. Interaction of dietary fiber with lipids—Mechanistic theories and their limitations. In *New Developments in Dietary Fibers*; Furda, I., Brine, C. J., Eds.; Plenum Press: New York, 1990; pp 67–82.

Gordon, D. T.; Besch-Williford, C. Action of amino polymers on iron status, gut morphology, and cholesterol level in the rat. In *Chitin, Chitosan, and Related Enzymes*; Zikakis, J. P., Ed.; Academic Press: Orlando, FL, 1984; pp 97–117.

Hirano, S.; Itakura, C.; Seino, H.; Akiyama, Y.; Nonaka, I.; Kanbara, N.; Kawakami, T. Chitosan as an ingredient for domestic animal feeds. *J. Agric. Food Chem.* **1990**, *38*, 1214–1217.

Ikeda, I.; Tomari, Y.; Sugano, M. Interrelated effects of dietary fiber and fat on lymphatic cholesterol and triglyceride absorption in rats. *J. Nutr.* **1990**, *119*, 1383–1387.

Ikeda, I.; Tomari, Y.; Sugano, M.; Watanabe, S.; Nagata, J. Lymphatic absorption of structured glycerolipids containing medium-chain fatty acids and linoleic acid, and their effect on cholesterol absorption in rats. *Lipids* **1991**, *26*, 369–373.

Jennings, C.D.; Boleyn, K.; Bridges, S. R.; Wood, P. J.; Anderson, J. W. A comparison of the lipid-lowering and intestinal morphological effects of cholestyramine, chitosan, and oat gum in rats. *Proc. Soc. Exp. Biol. Med.* **1988**, *189*, 13–20.

Kina, K.; Tamura, K.; Ishibashi, N. Use of 8-anilino-1-naphthalene sulfonate as a fluorescent indicator in colloid titration. *Jpn. Anal.* **1974**, *23*, 1802–1803.

Kobayashi, T.; Otsuka, S.; Yugari, Y. Effect of chitosan on serum and liver cholesterol levels in cholesterol-fed rats. *Nutr. Rep. Int.* **1979**, *19*, 327–334.

Nagyvary, J. J.; Falk, J. D.; Hill, M. L.; Schimdt, M. L.; Wilkins, A. K.; Bradbury, E. L. The hypolipidemic activity of chitosan and other polysaccharides in rats. *Nutr. Rep. Int.* **1979**, *20*, 677–684.

Nauss, J. L.; Thompson, J. L.; Nagyvary, J. The binding of micellar lipids to chitosan. *Lipids* **1983**, *18*, 714–719.

Sugano, M.; Fujikawa, T.; Hiratsuji, Y.; Hasegawa, Y. Hypocholesterolemic effects of chitosan in cholesterol-fed rats. *Nutr. Rep. Int.* **1978**, *18*, 531–537.

Sugano, M.; Fujikawa, T.; Hiratsuji, Y.; Nakashima, K.; Fukuda, N.; Hasegawa, Y. A Novel use of chitosan as a hypocholesterolemic agent in rats. *Am. J. Clin. Nutr.* **1980**, *33*, 787–793.

Sugano, M.; Ryu, K.; Ide, T. Cholesterol dynamics in rats fed *cis*- and *trans*-octadecenoic acid in the form of triglyceride. *J. Lipid Res.* **1984**, *25*, 474–485.

Sugano, M.; Watanabe, S.; Kishi, A.; Izume, M.; Ohtakara, A. Hypocholesterolemic action of chitosans with different viscosity in rats. *Lipids* **1988**, *23*, 187–191.

Sugano, M.; Goto, S.; Yamada, Y.; Yoshida, K.; Hashimoto, Y.; Matsuo, T.; Kimoto, M. Cholesterol-lowering activity of various undigested fractions of soybean protein in rats. *J. Nutr.* **1990**, *120*, 977–985.

Sugano, M.; Yoshida, K.; Hashimoto, M.; Enomoto, K.; Hirano, S. Hypocholesterolemic activity of partially hydrolyzed chitosans in rats. In *Advances in Chitin and Chitosan*: Brine, C. J., Sandford, P. A., Zikakis, J. P., Eds.; Elsevier Applied Science: London, 1992; pp 472–478.

Tanaka, K.; Aso, B.; Sugano, M. Biliary steroid excretion in rats fed soybean protein and casein or their amino acid mixture. *J. Nutr.* **1984**, *114*, 26–32.

Vahouny, G. V. Dietary fiber, lipid metabolism and atherosclerosis. *Fed. Proc.* **1982**, *41*, 2801–2896.

Vahouny, G. V.; Satchithanandam, S.; Cassidy, M. M.; Lightfoot, F. B.; Furda, I. Comparative effects of chitosan and cholestyramine on lymphatic absorption of lipids in the rat. *Am. J. Clin. Nutr.* **1983**, *38*, 278–284.

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