Relationship between structure and function of dietary fibre: a comparative study of the effects of three galactomannans on cholesterol metabolism in the rat

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Male adult rats were fed on diets containing 80 g/kg galactomannans with different galactose (G): mannose (M) ratios/kg. The galactomannans were compared with purified cellulose (Solkaflok) and the animals were also fed on a basal diet free from fibre. All diets contained cholesterol (10 g/kg) and sodium cholate (2 g/kg). The three galactomannans were fenugreek gum (1G:1M), guar gum (1G:2M) and locust-bean gum (1G:4M). In comparison with the fibre-free and Solkaflok diets, all three galactomannans lowered the concentrations of cholesterol in both liver and blood plasma. The galactomannans also decreased the rate of hepatic synthesis of cholesterol. Dietary galactomannans increased caecal volatile fatty acids, particularly propionic, increased the weight of the caecum and its contents and increased the amount of water in the faeces. The increase in propionic acid production was significantly related to a decrease in caecal pH, but not to changes in plasma cholesterol or hepatic cholesterol synthesis. These effects were significantly influenced by chemical composition and structure of the galactomannan; they were most evident when the proportion of galactose in the galactomannan was highest (i.e. fenugreek gum). The three galactomannans also differed markedly in their effects on the viscosity of the digesta, but the galactomannan which gave the highest viscosity was least effective in lowering plasma cholesterol. A separate experiment with perfused loops of small intestine in vivo showed that the most effective galactomannan, fenugreek gum, had no direct effect on cholesterol absorption.

Galactomannans: Dietary fibre: Cholesterol: Rat

The only forms of dietary fibre which have been convincingly shown to have the ability to lower plasma cholesterol concentrations are water-soluble fibre fractions. Pectins have been shown to lower plasma cholesterol in animal experiments and in human trials (Judd & Truswell, 1982, 1985). Similarly, guar gum has been shown to be effective in both animal and human studies (Jenkins *et al.* 1975, 1980; Simons *et al.* 1982). Xanthan gum (Osilesi *et al.* 1985), gum acacia (Sharma, 1985), locust-bean gum (Zavoral *et al.* 1983) and karaya gum (Behal *et al.* 1984) can also be effective, and the ability of oat bran to lower plasma cholesterol (Kirby *et al.* 1981) appears to be due to its high level of water soluble β-glucans (Chen *et al.* 1981; Oakenfull, 1988).

The ability of soluble fibre to lower plasma cholesterol concentrations appears to be due to either or both of two possible causes. First soluble fibre increases the viscosity of the digesta and increases the thickness of the unstirred layer in the small intestine. It might, therefore, be expected to inhibit uptake of cholesterol and bile acids (Gee *et al.* 1983). Secondly, having passed through the small intestine, soluble fibre is an excellent substrate for fermentation by the micro-organisms in the large bowel. The volatile fatty acids produced by fermentation enter the blood stream and appear to suppress hepatic cholesterol synthesis (Anderson & Bridges, 1981).

(a)

α-D-Gal$p$　　　　　　　　　　　　α-D-Gal$p$
　　1　　　　　　　　　　　　　　　1
　　$\downarrow$　　　　　　　　　　　　　　　$\downarrow$
　　6　　　　　　　　　　　　　　　6
β-D-Man$p$-(1→4)-β-D-Man$p$-(1→4)-β-D-Man$p$-(1→4)-β-D-Man$p$-(1→4)-

(b)

α-D-Gal$p$
　　1
　　$\downarrow$
　　6
β-D-Man$p$-(1→4)-β-D-Man$p$-(1→4)-β-D-Man$p$-(1→4)-β-D-Man$p$-(1→4)-
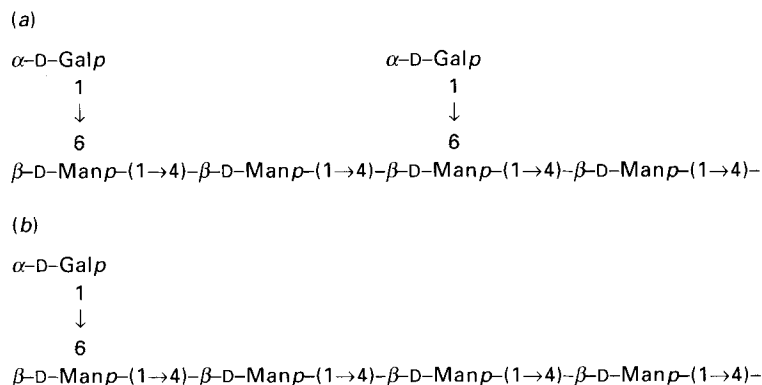
Fig. 1. Schematic diagram of the structure of the repeat unit of (a) guar (*Cyamopsis tetragonolobus*) gum and (b) locust bean (*Ceratonia siliqua*) gum.

A comparison of a series of galactomannans offers the opportunity to examine further these mechanistic possibilities and at the same time to investigate how small changes in the chemical structure of the polysaccharide might influence its metabolic effect.

The three galactomannans chosen were guar gum, locust-bean gum and fenugreek gum, all derived from the endosperm of seeds of Leguminosae species. Structurally they are based on a β-D-(1→4)-linked backbone of β-D-mannopyranosyl residues with side chains of single α-D-galactopyranosyl groups linked α-D-(1→6), as shown in Fig. 1 (Dea & Morrison, 1975). Depending on the botanical source, the galactomannans can have a range of galactose (G): mannose (M) ratios. For fenugreek gum (*Trigonella foenum-graecum*) the ratio is approximately 1G:1M, for guar gum (*Cyamopsis tetragonolobus*) approximately 1G:2M and for locust-bean gum (*Ceratonia siliqua*) approximately 1G:4M.

When included in the diets of cholesterol-fed rats, all three gums lowered plasma cholesterol compared with a control diet based on purified cellulose. More interestingly, the chemical structure influenced the magnitude of the effect.

MATERIALS AND METHODS
Animals and diets

Adult male Wistar rats (200–230 g body-weight) were used. They were randomly assigned to each diet group, six per group, and were housed in groups of three in cages with wire-mesh bottoms to minimize coprophagy. They were kept in a constant temperature environment ($25 \pm 1°$) with a 12 h cycle of light and dark. They were given free access to water and to the appropriate diet listed in Table 1 for a period of 2 weeks. The basal diet contained no dietary fibre.

Analytical procedures

Fresh faecal pellets (two per rat) were collected for moisture analysis on the last day of the treatment period. The animals were anaesthetized in an atmosphere of carbon dioxide. Blood was collected directly from the heart, and the liver and caecum were removed and weighed. The caecal contents were extruded from the caecum and thoroughly mixed with four parts of deionized water for measurement of caecal pH. To 1 ml of this suspension was added 0·1 ml sulphuric acid (500 ml/l) and the supernatant fraction after centrifugation was used for measurement of volatile fatty acids. These were measured with a Perkin-Elmer Sigma 3B gas–liquid chromatograph fitted with a glass column (length 2 m; i.d. 2 mm)

Table 1. *Composition of the diets (g/kg)*

Ingredient	
Maize starch	454
Casein	175·5
Whey powder	26·5
Sucrose	94·5
Maize oil	91
Dietary fibre*	80
Mineral mixture†	59·5
Vitamin mixture‡	4·5
Cholesterol	10
Sodium cholate	2
Choline	2·5

* Either guar (*Cyamopsis tetragonolobus*) gum, locust-bean (*Ceratonia siliqua*) gum, fenugreek (*Trigonella foenum-graecum*) gum or Solkaflok (a purified, non-fermentable fibre).

† Mineral content (g/kg): calcium carbonate 46, potassium dihydrogen phosphate 8, iodized sodium choride 3·5, magnesium sulphate 1, ferric ammonium citrate 0·7, manganese acetate 200 mg, zinc sulphate 60 mg, copper acetate 20 mg, ammonium molybdate 6 mg, chromium acetate 5 mg, sodium selenate 0·1 mg.

‡ Vitamin content (mg/kg): riboflavin 9, thiamin 6, pyridoxine 1·5, ascorbic acid 475, nicotinamide 79, sodium pantothenate 3·75, vitamin D 4500 I.U., vitamin A 18000 I.U.

packed with Chromosorb 101 and operated isothermally at 170°. Total plasma and high-density-lipoprotein (HDL)-cholesterol were measured using enzymic kits (Boehringer-Mannheim). Liver lipids were isolated using the extraction method of Bligh & Dyer (1959) and cholesterol measured in the non-saponifiable fraction using the methods of Hood (1987) and Rudel & Morris (1973). Thin slices of liver (100–150 mg) were incubated in 20 ml vials containing 3 ml Krebs–Ringer buffer (Ca^{++}-free) as described by Hood (1990).

Viscometry

The rheological properties of aqueous solutions of the three galactomannans were studied using a Bohlin VOR Rheometer with cone and plate geometry. All measurements were made at 37°. Measurements were made of the variation of apparent viscosity with shear rates within the range 0·02–15/s.

Absorption from loops of small intestine

The animals were male Wistar rats of body-weight 200–250 g maintained on a commercial diet containing 230 g protein/kg and 50 g crude fat/kg (Allied Feeds, Rhodes, NSW). They were starved of food overnight and anaesthetized with intraperitoneal sodium pentobarbital (60 mg/kg body-weight). The abdomen was opened by midline incision and the entire length of the jejunum and ileum divided into two sections, 'upper' and 'lower', of approximately equal length. Inflow cannulas were fitted and the lumen of each section was washed with isotonic saline (9 g sodium chloride/l) at 37°. Outflow cannulas were then fitted and the sections replaced inside the abdominal cavity which was closed using surgical clips. Isotonic solutions, pH 7·4 at 37°, were circulated separately through the lumen of each section at 2 ml/min for 60 min. The solution for the upper loop initially contained either ³H-labelled glucose (10 mM) or a micellar solution of ³H-labelled cholesterol (0·1 mM) with sodium taurocholate (10·0 mM), oleic acid (1·2 mM) and monoolein (0·6 mM). The solution for the lower loop initially contained ³H-labelled glucose (10 mM) or ³H-labelled sodium taurocholate (4 mM). All solutions also contained ¹⁴C-labelled CrEDTA to monitor absorption or loss of water. Samples of the circulating solution were taken at 20 min

Table 2. *Effects of three galactomannans derived from the seed endosperm of Leguminosae spp. on caecal contents and faeces of rats*

(Means with their standard errors)

Dietary treatment*	Live wt (g)		Caecal contents (g)		Caecal wt (g)		Caecal pH		Faecal water (g/kg)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Basal	242[a]	6	1·37[a]	0·10	1·59[a]	0·09	7·69[a]	0·07	414[a]	12
Solkafiok†	228[a,b]	11	1·36[a]	0·24	1·34[a]	0·17	7·69[a]	0·07	494[b]	23
Fenugreek (*Trigonella foenum-graecum*)	210[b,c]	6	2·85[b]	0·26	2·19[b]	0·12	6·64[b]	0·09	629[c]	18
Guar (*Cyamopsis tetragonolobus*)	220[a,c]	8	3·46[c]	0·27	2·52[c]	0·26	6·38[c]	0·05	651[d]	7
Locust bean (*Ceratonia siliqua*)	196[c]	5	3·03[c]	0·22	2·58[c]	0·14	6·14[d]	0·07	621[c]	33

[a,b,c,d] Values in vertical columns with unlike superscript letters were significantly different ($P < 0.05$).

* For details, see p. 218 and Table 1.

† Purified cellulose.

intervals for radioactive counting. Rates of absorption of cholesterol, taurocholate and glucose were calculated with appropriate corrections for water flux.

Statistical analyses

Differences between means were tested using a two-sided t test, taking $P < 0.05$ as the criterion of significance. Relationships between variables were examined using correlation coefficients between pairs (Snedecor & Cochran, 1989) calculated using MINITAB.

RESULTS

Effects on the body-weight, caecum and faeces

The average weights of the rats were similar for each group at the commencement of the dietary treatment. At the end of the 2-week treatment weight gains and final body-weights (Table 2) were similar for the three groups given galactomannan, intermediate for rats given Solkaflok and highest for rats on the basal diet. Feeding the galactomannans caused large increases in the weight of the contents of the caecum and also in the weight of the caecum after the contents had been expressed (Table 2).

The pH of the caecal contents was lower for the galactomannan-fed animals than for those fed on the basal diet or Solkaflok, indicating more intensive microbial activity with production of volatile fatty acids.

The galactomannans all increased the water content of the faeces compared with the basal diet or Solkaflok. The differences between the individual galactomannans were small, however, and not statistically significant.

Liver and plasma cholesterol concentrations

The results presented in Table 3 show that the galactomannans strongly influenced the levels of cholesterol in liver and plasma. In each case the levels were considerably lower than when the animals were fed on the basal (fibre-free) diet or Solkaflok. In addition, there were smaller but mostly significant differences between the galactomannans. The higher the density of galactose side chains the greater the effect. Thus, the magnitude of the cholesterol-lowering effect was consistently in the order fenugreek > guar > locust bean.

The only difference observed between the fibre-free and Solkaflok diets was in the small increase in the total plasma cholesterol, particularly the non-HDL component, in response to dietary Solkaflok.

Rates of cholesterol synthesis

Rates of cholesterol synthesis were estimated in the liver and small intestine from [^{14}C]mevalonate (Hood, 1990). These results are shown in Table 4. The galactomannans had a dramatic effect on hepatic cholesterol synthesis which was lowered by a factor of approximately two when compared with the basal and Solkaflok diets. Again there were smaller but significant differences between the effects of the individual galactomannans. The magnitude of inhibition of cholesterol synthesis was in the same order as the lowering of plasma and liver cholesterol: fenugreek > guar > locust bean.

Caecal volatile fatty acids

Acetic, propionic and butyric acids were the major fermentation products in the caecum of rats fed on either the basal or Solkaflok diets (Table 5). When galactomannan replaced Solkaflok in the diet the proportions of the different volatile fatty acids were altered; in particular, propionic acid increased and butyric acid was detected only in trace amounts. Propionate production was greatest when the rats were fed on locust-bean gum which had the least number of galactose side chains. The effects of diet on the proportions of the volatile fatty acids are shown in Fig. 2.

Table 3. *Effects of three galactomannans derived from the seed endosperm of Leguminosae spp. on total liver lipids and the body pool of cholesterol of rats*

(Means with their standard errors)

Dietary treatment*	Liver lipids (g/kg)		Liver cholesterol (mg/liver)		Serum cholesterol (mM)			
					Total		HDL	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Basal	10.5^a	0.8	300^a	23	3.90^a	0.07	2.38^a	0.04
Solkaflok†	10.7^a	1.6	256^a	141	4.60^b	0.34	2.23^a	0.06
Fenugreek (*Trigonella foenum-graecum*)	4.9^b	0.4	75^b	15	3.16^c	0.09	2.37^a	0.06
Guar (*Cyamopsis tetragonolobus*)	4.6^b	0.3	76^b	11	3.26^c	0.09	2.14^a	0.08
Locust bean (*Ceratonia siliqua*)	5.5^c	0.6	113^c	18	3.46^d	0.12	2.44^a	0.06

[a,b,c,d] Values in vertical columns with unlike superscript letters were significantly different ($P < 0.05$).

HDL, high-density lipoprotein.

* For details, see p. 218 and Table 1.

† Purified cellulose.

Table 4. *Effects of three galactomannans derived from the seed endosperm of Leguminosae spp. on the relative rates of hepatic synthesis of total lipid and cholesterol and intestinal synthesis of cholesterol from mevalonate (nmol mevalonate converted into product in rats)*

(Means with their standard errors)

Dietary treatment*	Total lipid (nmol/liver per h)		Cholesterol (nmol/liver per h)		Cholesterol (nmol/g intestine per h)	
	Mean	SE	Mean	SE	Mean	SE
Basal	762^a	72	103^a	9	0.44^a	0.08
Solkaflok†	697^a	81	90^a	7	0.36^a	0.05
Fenugreek (*Trigonella foenum-graecum*)	463^b	59	39^b	5	0.42^a	0.09
Guar (*Cyamopsis tetragonolobus*)	$523^{b,c}$	68	44^b	3	0.22^b	0.02
Locust bean (*Ceratonia siliqua*)	585^c	59	65^c	9	0.37^a	0.04

[a,b,c] Values in vertical columns with unlike superscript letters were significantly different ($P < 0.05$).

* For details, see p. 218 and Table 1.

† Purified cellulose.

Table 5. *Effects of three galactomannans derived from the seed endosperm of Leguminosae spp. on caecal volatile fatty acid concentration of rats*

(Means with their standard errors)

| | Volatile fatty acids (μmol/g) | | | | | |
| | Acetic | | Propionic | | Butyric | |
Dietary treatment*	Mean	SE	Mean	SE	Mean	SE
Basal	66.0[a]	3.8	20.8[a]	1.2	3.7[a]	0.2
Solkaflok†	45.2[b]	1.0	14.0[b]	0.8	4.4[a]	0.7
Fenugreek (*Trigonella foenum-graecum*)	59.7[a]	2.6	49.5[c]	2.8	tr	
Guar (*Cyamopsis tetragonolobus*)	52.8[b]	3.4	57.2[d]	1.4	tr	
Locust bean (*Ceratonia siliqua*)	50.5[b]	2.7	67.9[e]	2.3	tr	

[a,b,c,d,e] Values in vertical columns with unlike superscript letters were significantly different ($P < 0.05$).
tr, trace.
* For details, see p. 218 and Table 1.
† Purified cellulose.

Metabolic correlations

Correlation coefficients were calculated for pairs of biochemical measurements using the pooled data from the three galactomannan treatment groups. Galactomannans in the diet caused a reduction in caecal pH (Table 2) which was significantly correlated ($P < 0.01$) with the increase in propionic acid production, but not with acetic acid production (Table 6). Plasma cholesterol and the rate of hepatic cholesterol synthesis were significantly correlated ($P < 0.01$); however, neither of these two quantities were related to caecal propionate production.

Viscosity

The viscosities of aqueous solutions of the three galactomannans were compared at two different concentrations (5 and 10 g/kg) and over a broad range of rates of shear. The results are shown in Fig. 3. The solutions are non-newtonian and all three curves show the decrease in apparent viscosity with increasing shear rate typical of food gums (Bourne, 1982). Contrary to a previous report (Bourne, 1982) we were unable to detect a yield stress at low shear rates for any of the samples.

More relevant to the present study, the results presented in Fig. 3 show the relative viscosities of the three galactomannans at shear rates within the range encountered during passage of digesta through the small intestine. Taking the radius of the rat small intestine to be approximately 3 mm and the maximum flow-rate for the digesta to be 2 ml/min., the shear rate ranges from zero at the centre to approximately 2/s at the wall (Sherman, 1970). Within this range the results show that guar gum is the most viscous and fenugreek gum the least viscous of the three galactomannans.

The results presented are comparative among treatment groups for the perfused intestine which behaves like a tube of constant radius with a steady flow; in our experiments, 2 ml/min. However, in vivo, due to waves of contractions migrating along the length of the intestine, the diameter of the gut wall will vary. Intermittent stirring and changing peristaltic pressures may result in shear rates at the point of maximum constriction that are higher than the theoretical values quoted in Fig. 3.
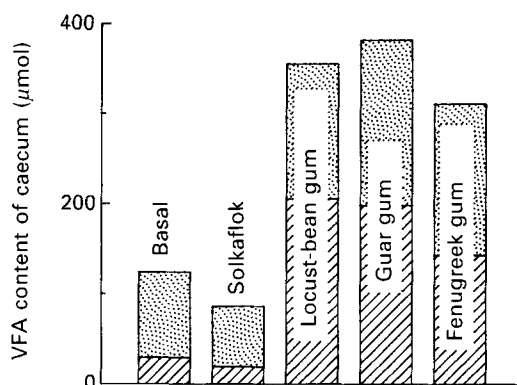
Fig. 2. Effect of dietary galactomannans derived from the seed endosperm of Leguminosae spp. on the caecal content of acetic (☐) and propionic (▨) acids (μmol/caecum) in rats. VFA, volatile fatty acids. Solkaflok (purified cellulose), locust bean (*Ceratonia siliqua*), fenugreek (*Trigonella foenum-graecum*) and guar (*Cyamopsis tetragonolobus*) gum were included at 80 g/kg diet. For details of diets, see p. 218 and Table 1.

Table 6. *Selected correlation coefficients for biochemical measurements in rats from the three groups fed on galactomannans derived from the seed endosperm of Leguminosae spp.*

(Correlation coefficients numerically greater than 0·56 are significant ($P < 0.01$))

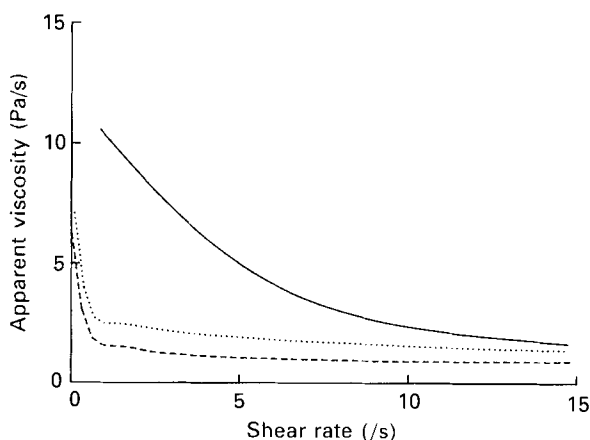| Comparison	Correlation coefficient
Caecal pH *v*. caecal propionate	−0·79
Caecal pH *v*. caecal acetate	−0·38
Plasma cholesterol *v*. cholesterol synthesis	0·61
Plasma cholesterol *v*. caecal propionate	0·26
Cholesterol synthesis *v*. caecal propionate	0·25

Fig. 3. Apparent viscosity *v*. shear rate for guar (*Cyamopsis tetragonolobus*) gum (——), locust bean (*Ceratonia siliqua*) gum (...) and fenugreek (*Trigonella foenum-graecum*) gum (---) at a concentration of 10 g/kg. Rates of shear within the rat small intestine would be approximately within the range zero to 2/s.

Effect of fenugreek gum on cholesterol absorption from the small intestine in vivo

In a separate study, isotonic solutions containing fenugreek gum and cholesterol were circulated through loops of small intestine in vivo in the rat. Fenugreek gum had no significant effect on the rate of absorption of cholesterol, as shown in Table 7. It also had

no effect on the rate of absorption of taurocholate but there was a small, but statistically significant, inhibition of absorption of glucose in both the upper and lower loops.

DISCUSSION

Influence of chemical structure on cholesterol-lowering activity

The ability of guar gum to lower plasma and liver cholesterol concentrations is now well established (Jenkins *et al.* 1975, 1980; Simons *et al.* 1982). Locust-bean gum has also been shown to lower plasma cholesterol (Zavoral *et al.* 1983) as have fenugreek seeds (but not the isolated gum; Sharma 1986). So the powerful cholesterol-lowering activity of the three galactomannans in the present study is not in itself unexpected. What is of interest is that the magnitude of the effect is influenced by subtle changes in the chemical structure of the galactomannan.

The galactomannans all have the same β-D-mannan backbone. They differ only in the proportion of D-galactosyl side groups per D-mannose residue, as shown in Fig. 1. Guar gum, for example, has approximately twice as many D-galactosyl side groups per D-mannose as locust-bean gum (Dea & Morrison, 1975). The side groups appear to be arranged along the backbone in blocks rather than randomly, but this fine structure is not well defined. Studies with locust-bean gum have indicated batch-to-batch variations, possibly resulting from different extraction and purification procedures (Dea & Morrison, 1975).

The results presented in Tables 2 and 3 suggest that the cholesterol-lowering activity of the galactomannans depends on the D-galactose : D-mannose ratio. The greater this ratio, the greater the activity. This effect appears to be perfectly consistent for different variables. Thus, the magnitude of the lowering of total plasma cholesterol was in the order: fenugreek > guar gum > locust-bean gum (Table 2); the magnitude of suppression of hepatic cholesterol synthesis was in the same order (Table 3).

The mechanism of cholesterol-lowering by galactomannans

Do these results shed any light on the mechanism of cholesterol-lowering by galactomannans? Three possible mechanisms currently seem plausible:

(1) Soluble fibre increases the viscosity of the digesta and this may inhibit the absorption of cholesterol from the small intestine and also the reabsorption of bile acids from the terminal ileum (Gee *et al.* 1983).

(2) Soluble fibre is an excellent substrate for the micro-organisms in the large bowel. The fermentation that takes place there produces volatile fatty acids (Smith & Bryant, 1979) which enter the blood stream and may influence hepatic synthesis of cholesterol (Ide *et al.* 1978; Anderson & Bridges, 1981).

(3) Fibre may adsorb bile acids in the small intestine (Eastwood & Hamilton, 1968; Kritchevsky & Story, 1974). Adsorbed bile acids would be diverted from the enterohepatic cycle, lost by faecal excretion, and this loss offset by conversion of cholesterol into bile acids by the liver (Heaton, 1972).

Our results argue against the first of these suggestions. Viscosity does not seem to be a significant factor. The magnitude of the cholesterol-lowering effect of the three galactomannans was in the opposite order to their effect on viscosity. The most viscous (guar) had less effect on plasma cholesterol than the least viscous (fenugreek). Also, in the separate study of the effect of fenugreek gum on absorption from perfused loops of small intestine, the gum had no significant effect on the rate of absorption of cholesterol (Table 7). The gum did, however, significantly inhibit the absorption of glucose.

These results are supported by the findings of Topping *et al.* (1988) who compared the

Table 7. *Effect of fenugreek (Trigonella foenum-graecum) gum on the rate of intestinal absorption* of cholesterol, glucose and taurocholate, in vivo, in the rat*

(Means with their standard errors)

Dietary treatment†	Upper loop						Lower loop					
	Cholesterol		Glucose		Taurocholate			Glucose	
	Mean	SE	Mean	SE			Mean	SE	Mean	SE
Control	0.431^a	0.024	2.96^a	0.08			25.7^a	2.4	2.82^a	0.15
Fenugreek (*Trigonella foenum-graecum*)	0.395^a	0.033	2.59^b	0.18			26.5^a	0.80	1.97^b	0.30

a,b Values in vertical columns with unlike superscript letters were significantly different ($P < 0.05$).

* μmol/s per cm length of small intestine.

† For details, see p. 218 and Table 1.

effects in the rat of feeding methyl celluloses of different grades of viscosity. In their experiments also, viscosity had no influence on plasma cholesterol concentrations, but did influence plasma glucose. Moreover, gum acacia has no significant effect on viscosity yet produced a 10·4 % reduction in plasma cholesterol when included in the diet of a group of hypercholesterolaemic men (Sharma, 1985).

Our results are also inconsistent with the second proposed mechanism. The cholesterol-lowering activity of the galactomannans appears not to be directly related to their susceptibility to fermentation in the large bowel. The galactomannan with the greatest cholesterol-lowering activity had least effect on caecal pH and least effect on the size of the caecum and mass of caecal contents (Table 2). But, at the same time, the three galactomannans did produce substantial reductions in the rate of hepatic synthesis of cholesterol. Moreover, the magnitude of the reduction was in each case proportional to the lowering of plasma cholesterol.

Another point to be considered in this connexion is that the concentration of propionate which was found to inhibit cholesterol synthesis in vitro was ten to fifteen times higher than that actually found in the portal vein in vivo during fermentation of soluble fibre in the large bowel (Illman *et al.* 1988). Although propionic acid production was significantly correlated with caecal pH in rats fed on galactomannan, it was not related to plasma cholesterol concentration or to the rate of hepatic synthesis of cholesterol. Consequently the effects of soluble fibre on plasma cholesterol appear not to be related to propionate production. Cheng *et al.* (1987) also reported no relationship between individual volatile fatty acids and either blood glucose or plasma lipids.

The third mechanism, that of adsorption of bile acids, is supported by the finding that, in the rat, cholesterol-lowering by oat bran is accompanied by increased faecal excretion of bile acids (Illman & Topping, 1985). However, our gut perfusion study (Table 7) showed that there was no effect from fenugreek gum on intestinal absorption of taurocholate. Moreover, cholesterol-lowering by pectin is also accompanied by increased faecal excretion of bile acids (Kay & Truswell, 1977) but adsorption of bile acids onto pectin appears to be physically impossible at gut pH because both molecules are negatively charged (Oakenfull & Sidhu, 1984). Pectin at concentrations up to 30 g/l did not affect the rate of cholate diffusion through cellulose membranes (Oakenfull & Sidhu, 1984). This concentration is not likely to be exceeded in the digesta of human subjects ingesting soluble dietary fibres. Judd & Truswell (1982) did not find any significant differences in plasma cholesterol or faecal steroid excretion in human subjects fed on either low- or high-methoxyl pectins. The degree of hydrophobicity of these pectins would strongly influence any capacity they might have to absorb bile acids. Thus, intestinal absorption and binding of bile acids or cholesterol by soluble fibres does not satisfactorily explain their hypocholesterolaemic effect.

Thus, our results are not wholly consistent with any of the possible mechanisms so far proposed to explain how soluble fibre influences cholesterol metabolism. It may be relevant that rats fed on guar gum or carboxymethylcellulose (another soluble fibre) show significant adaptive differences when compared with animals fed on similar quantities of insoluble cellulose (Johnson & Gee, 1986). There were substantial morphological changes in the large bowel and changes in the activities of some brush-border enzymes. This is confirmed in part in our study which showed large increases in the size of the caecum in response to feeding the galactomannans. Also, again as observed in our study, Johnson & Gee (1986) found significant quantitative differences between the effects of their two types of soluble fibre. In another study, using mice (Stanley & Newsholme 1985), guar gum was shown to increase the activity of a number of key enzymes in carbohydrate and lipid metabolism by the liver, despite the fact that the guar gum remains in the gastrointestinal tract. The link may possibly be via the gastrointestinal hormones since guar gum reduces

the secretion of gastric inhibitory polypeptide and gut glucagon-like immunoreactivity in human subjects (Morgan *et al.* 1979).

Thus, the mechanism by which soluble fibre lowers plasma cholesterol is complex and remains poorly understood.

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