The degradation of guar gum by a faecal incubation system

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1. Homogenized and diluted faeces (50 g/l) from one human source were incubated with the complex plant polysaccharide, guar gum, to investigate the degradation of viscous polysaccharides by intestinal bacteria.

2. Incubation of the faecal homogenate with guar gum produced a rapid decrease in viscosity and in pH, accompanied by the release of hydrogen.

3. No changes in viscosity or pH were observed and there was no production of H_2 gas when guar gum was incubated with autoclaved faecal homogenate (20 min, $1{\cdot}03 \times 10^5$ Pa).

4. A bacteria-free filtrate of faeces was prepared by centrifuging the faecal homogenate (2400 ***g*** for 100 min) followed by filtration through a Seitz filter and then a millipore filter (size 0·45 μm). Incubating this with guar gum produced a slow decrease in viscosity, but no significant change in pH and no generation of H_2.

5. Our results show that guar gum can be fermented by human colonic bacteria and suggest the possibility of predigestion by extracellular free enzymes.

Storage polysaccharides, such as guar gum, pectin and gum tragacanth are used clinically to treat diabetes mellitus, obesity and dumping syndrome (Jenkins *et al.* 1977; Leeds *et al.* 1981; Krotkiewski, 1984). They are not broken down by normal human digestive enzymes and form viscous gels in the stomach and small intestine which delay the absorption of glucose and other nutrients from the small intestine by several mechanisms (Holt *et al.* 1979; Morgan *et al.* 1979; Johnson & Gee, 1980; Blackburn *et al.* 1984).

When they reach the large intestine, viscous polysaccharides may be broken down by the rich bacterial flora. The extent of colonic degradation of many polysaccharides is unknown. Some polysaccharides may not be broken down at all, others may be partially degraded leading to a decrease in viscosity, and others may be completely fermented to volatile fatty acids (VFA), and gases such as hydrogen and carbon dioxide.

Bacterial fermentation of viscous polysaccharides has several clinical implications: gas production may result in distension and flatulence (Jenkins *et al.* 1980); the reduction in pH may alter various aspects of colonic metabolism, for example the deconjugation of bile acids (Macdonald *et al.* 1978); and the loss of the water-holding properties of the viscous gel may decrease its potential effect on stool bulk and bowel function, although this action may be offset by the increased microbial mass stimulated by the additional carbohydrate source (Stephen & Cummings, 1980).

Studies of the degradation of viscous polysaccharides by colonic bacteria in vitro may help to explain the clinical actions of polysaccharides that are currently used in therapy, and to predict the possible clinical application of new polysaccharide substances. This study has used a faecal incubation system to investigate how a mixed population of faecal bacteria digests guar gum. It is assumed that the bacteria in fresh faeces are the same as the bacteria of the colon and rectum. Studies on sudden death victims have shown that the composition of the lumenal flora remains relatively constant from ascending colon to rectum (Moore *et al.* 1978) and that the flora of the mucosa is as complex as that in faeces (Croucher *et al.* 1983).

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Table 1. *Composition of the medium*

Component	Concentration	Sterilization procedure
Sodium chloride	60 mM	Dissolved in distilled water, then autoclaved at $1{\cdot}03 \times 10^5$ Pa for 20 min
Sodium bicarbonate	40 mM	
Potassium chloride	10 mM	
Tryptone*	10 g/l	
Hemin†	5×10^{-5} g/l	Dissolved in sodium hydroxide solution (100 g/l) then filter sterilized and added to the above

* Oxoid Ltd, Basingstoke.
† BDH Chemicals Ltd, Poole, Dorset.

METHODS

Guar gum (5 g/l) was incubated with a faecal homogenate that was untreated or had been filtered to remove bacteria and leave extracellular enzymes or had been autoclaved to destroy the bacteria and the enzymes. Changes in viscosity and pH during the incubation were measured, and gases produced were collected for analysis.

Experimental procedures

Powdered guar gum (Hercules Powder Co., Erith, Kent) was made up as a solution (10 g/l) in sterile medium (Table 1). To model conditions at the ileo-caecal transition the electrolyte composition of the medium was based on ileostomy effluent and the pH used was at the upper end of the pH range seen in the terminal ileum by Bown *et al.* (1974).

Fresh human faeces were collected from a healthy donor, who was taking a normal Western diet and had not taken antibiotics for 6 months. A 10 g sample was homogenized in 200 ml of sterile medium (50 g/l) under anaerobic conditions created by vigorous bubbling with nitrogen–CO_2 (0·95:0·05, v/v).

The faecal homogenate was then divided into three: (*a*) a portion was left untreated and so contained bacteria and free enzymes in the faecal water; (*b*) a portion was centrifuged at 2400 ***g*** for 100 min, Seitz filtered and then passed through a millipore filter (pore size 0·45 μm) at room temperature, to exclude bacteria but leave the free enzymes; (*c*) a portion was autoclaved at $1{\cdot}03 \times 10^5$ Pa (equivalent to 120°) for 20 min so that bacteria were killed and enzymes denatured. Inoculations were made into sterile 30 g universal bottles in an anaerobic cabinet (Don Whitley, Baildon, W. Yorks) that contained N_2–CO_2–H_2 (0·8:0·1:0·1, v/v).

Each set of experiments consisted of seven incubations; they comprised a test incubation for each of the three faecal preparations (untreated, filtered and autoclaved) with guar gum, a control incubation for each of the three faecal preparations in which guar gum was replaced by sterile medium, and one control incubation in which sterile medium replaced the faecal preparation. These mixtures were made up in duplicate (one set of seven bottles was analysed within 40 min to give the initial readings and the other set was incubated at 37° for 21 h and then analysed to give the final readings).

Measurements

The viscosity was measured with an Otswald viscometer (Mark, 1945), capillary bore 2 mm,

Table 2. *Changes in viscosity of the cultures on incubation at 37° for 21 h*
(Mean values with their standard errors)

Components		Viscosity (mPas)				Statistical significance of difference: $P <$
		Initial		Final		
		Mean	SE	Mean	SE	
Guar gum alone	Control	173·6	12·4	150·0	13·9	NS
Untreated faeces	Control	1·9	0·0	2·0	0·0	NS
Untreated faeces	With guar gum	43·3	8·0	2·2	0·0	0·001
Filtered faeces	With guar gum	174·4	35·2	13·3	3·2	0·001
Autoclaved faeces	With guar gum	216·2	17·8	191·0	11·0	NS

NS, not significant.

calibrated with olive oil at the same temperature as the sample to be tested (room temperature). Viscosity was calculated from the formula:

$$V_s = \frac{t_s \times d_s \times V_o}{t_o \times d_o},$$

where V is viscosity, t is time, d is density, o is olive oil and s is sample.

This method is not valid for non-Newtonian fluids, but since guar gum is thought to act as a Newtonian fluid up to a concentration of 5 g/l (Goldstein *et al.* 1973), the maximum concentration found in any of the cultures, the results are adequate for comparisons within the study.

The pH was measured before and after incubation using a pH electrode connected to a pH meter (Model 10; Corning Eel, Medfield, MA, USA).

Gases produced during the incubation were collected in a greased glass syringe. This was connected to the small gas space above the incubation mixture via a three-way tap and a needle pushed through a small hole in the metal cap and through the rubber seal beneath. The syringe contents were analysed for H_2 concentration using a breath H_2 monitor (Hansatech, King's Lynn) calibrated from 0 to 96 μl/l with air and a standard gas mixture (GMI, Renfrew, Scotland).

Statistical analysis

Results were analysed statistically by Student's paired t test.

RESULTS

Untreated faeces

A very rapid decrease in viscosity to about 25% of the value for the guar gum control ($P < 0{\cdot}01$) was observed within 40 min of mixing guar gum with the untreated faeces (Table 2). Incubation for 21 h caused a further reduction in viscosity ($P < 0{\cdot}01$) to the same level as that of the faecal suspension without guar gum and significantly lower than the corresponding value for the guar gum control ($P < 0{\cdot}01$). This reduction in viscosity was accompanied by the production of H_2 gas and a fall in pH to values lower than the initial value and either control ($P < 0{\cdot}01$) (Table 3).

Control cultures containing untreated faeces but no guar gum had a low viscosity that

Table 3. *Changes in pH of the cultures and hydrogen concentration produced on incubation at 37° for 21 h*

(Mean values with their standard errors)

Components		pH Initial Mean	pH Initial SE	pH Final Mean	pH Final SE	Statistical significance of difference: $P <$	Mean H_2 concentration (ml/l) Mean	Mean H_2 concentration (ml/l) SE
Guar gum alone	Control	8·60	0·10	8·29	0·08	0·05	0	
Untreated faeces	Control	8·12	0·26	6·91	0·24	0·001	26·8	18·8
Untreated faeces	With guar gum	8·14	0·21	6·18	0·10	0·001	63·8	31·7
Filtered faeces	With guar gum	8·39	0·21	8·00	0·10	NS	0	
Autoclaved faeces	With guar gum	8·75	0·30	8·60	0·30	NS	0	

NS, not significant.

did not change during incubation ($P > 0·05$) (Table 2). There was, however, a reduction in the pH of the cultures and some H_2 was generated, although the changes were much less than those observed when guar gum was present ($P < 0·01$) (Table 3).

No significant pH reduction or H_2 production occurred in the control mixtures that did not contain the untreated faeces (Table 3).

Filtered faeces

When bacteria were excluded from the faecal homogenate by filtration, the initial viscosity value of the guar gum with filtered faeces was not significantly different from that of the guar gum control (Table 2). The viscosity of the incubated mixture, however, was reduced to about 9% of that of the guar gum control ($P < 0·01$); significantly lower than its original value ($P < 0·01$) (Table 2) but still higher than the viscosity of the guar gum incubated with untreated faeces ($P < 0·01$).

The reduction in viscosity observed on incubating guar gum with filtered faeces was not accompanied by a significant fall in pH or the production of H_2 (Table 3).

Autoclaved faeces

When the faecal homogenate was heat-sterilized to destroy both bacteria and enzymes, the results were little different from control measurements. The initial viscosity of the mixture was in fact slightly higher than that of the guar gum control ($P < 0·05$) (Table 2). This is probably due to a loss of liquid from the faeces during sterilization, making the actual concentration of guar gum in the mixture slightly greater than 5 g/l. The final viscosity value, however, was not significantly different either from its corresponding initial value or from the final value of the guar gum control.

Incubation of guar gum with autoclaved faeces did not cause the pH to fall ($P > 0·05$) and did not generate any H_2 gas (Table 3).

DISCUSSION

Guar gum was rapidly degraded by a homogenate of faeces; the viscosity was reduced by 75% within 40 min and the pH also fell significantly. After incubation for 21 h, the viscous properties of the mixture were no different from those of the homogenized faeces control,

the pH had fallen significantly by an average of 1·7 pH units and a high concentration of H_2 gas had been produced. These changes were almost certainly caused by bacterial action, since they did not occur during incubation of guar gum with autoclaved faeces. The smaller reduction in pH and release of H_2 observed when the untreated faecal preparation was incubated without guar gum can probably be explained by bacterial fermentation of substrates already present in the faeces, such as mucoproteins (Hoskins & Boulding, 1981) and unabsorbed polysaccharides from foodstuffs. Tryptone present in the medium may also be digested, but experiments with cultures of single species of bacteria suggest that whilst H_2 can be produced in this way, changes in pH (presumably reflecting production of VFA) cannot (J. Tomlin, C. A. Edwards, B. I. Duerden and N. W. Read, unpublished results).

These experiments have confirmed that guar gum can be fermented by faecal bacteria, resulting in a total abolition of its viscous properties. This result could explain why the contents of the large intestine of rats fed on guar gum are no more viscous than those of controls (Blackburn & Johnson, 1981) and why guar gum has a variable effect on faecal bulking in man (Cummings *et al.* 1978) but produces a large amount of gas (Jenkins *et al.* 1980).

The incubation of guar gum with a bacteria-free filtrate of faeces produced a significant reduction in viscosity but, in contrast to the findings with untreated faeces, there was no change in pH and no generation of H_2. This observation suggests that the presence of bacteria is necessary for the pH reduction and release of H_2 but that the viscosity reduction can be produced by extracellular enzymes in the filtrate. A reduction in viscosity caused by an interaction between polysaccharide and protein in the faecal filtrate seems unlikely because there is little or no interaction between proteins and non-ionic gums such as guar gum (Ganz, 1974) and any coacervation would tend to increase, rather than reduce, the observed viscosity. Production of extracellular enzymes in response to viscous polysaccharides does occur in isolated strains of colonic anaerobes (Salyers *et al.* 1978) and an extracellular enzyme that reduces the viscosity of guar gum has been observed in cultures of *Bacteroides ovatus*, although there was no simultaneous monitoring of pH or gas production in that study (Balascio *et al.* 1981). The enzymes in the filtrate actually could be secreted by the bacteria or they could be released by cell lysis either during slow passage through the colon or during preparation of the bacteria-free filtrate. The viscous properties of guar gum in solution may make it difficult for bacteria to gain access to the polysaccharide; it is possible that predigestion by extracellular enzymes could render the gum less viscous and so more readily accessible to bacteria. It is likely that guar gum is broken down by a number of enzymic stages before fermentation occurs; extracellular enzymes may reduce the viscosity and generate high-molecular-mass products, bacteria-associated mannanases clip the backbone at every 3rd to 7th unit and, finally, bacterial α-galactosidase (*EC* 3.2.1.22) and β-mannosidase (*EC* 3.2.1.25) break apart the remaining sugar molecules and allow fermentation to proceed (Emi *et al.* 1972; Prizont *et al.* 1976; Gherhardini & Salyers, 1982).

Salyers *et al.* (1977*a*, *b*) found that a surprisingly small proportion (twenty-nine of 286 strains) of anaerobic colonic bacteria tested could ferment guar gum in isolation. It is possible that these are the strains that can produce extracellular enzymes, and that many other strains could have fermented the guar gum if the preliminary digestion stage had been performed.

Production of extracellular enzymes is often induced by the presence of their substrate (Salyers *et al.* 1978), suggesting that the bacterial species in the faecal samples used in the present study had been exposed to the linkages present in guar gum *in situ*. This is possible since guar gum is a common food additive in concentrations up to 20 g/kg (Bowes & Church, 1975), and polysaccharides with β-1 → 4 manno-pyranosyl and α-1 → 6

galacto-pyranosyl linkages are present in many beans (especially soya) and peas (Royal College of Physicians of London, 1980).

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