Intestinal microflora and gastrointestinal adaptation in the rat in response to non-digestible dietary polysaccharides

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1. A comparison was made of the effect of a fibre-free diet and diets containing non-digestible polysaccharides on rat caecal and colonic physiology and microflora.

2. All polysaccharide-containing diets led to enlargement of the caecum and colon, associated with increased weight of contents, and of tissue. Carboxymethylcellulose (CMC) had the most marked effect and animals given this also had watery faeces.

3. The density of bacteria in the caecum and colon varied significantly with diet and the proportion of aerobic bacteria in the flora was increased by the CMC diet.

4. In vitro, CMC and hydroxypropylmethylcellulose were poorly fermented.

5. There was a high correlation (caecum *r* 0·93; colon *r* 0·94) between tissue weight and wet weight of organ contents but no correlation with bacterial density, number of bacteria per organ, moisture content or short-chain fatty acid content.

6. It is concluded that caecal and colonic enlargement is due to tissue hypertrophy in response to increased bulk of contents, irrespective of the nature of that bulk which varies with diet; it is unlikely that short-chain fatty acids or other microbial metabolites are the stimulus for the trophic response seen when non-digestible dietary polysaccharides are fed to rats.

It is well established that non-digestible components of the diet (that is, not digested by mammalian enzymes) influence the growth and function of the gastrointestinal organs in laboratory animals (Leegwater *et al.* 1974; Wang, 1979; Elsenhans *et al.* 1981; Rowland & Walker, 1983; Konishi *et al.* 1984). The hypertrophic effect of carbohydrate polymers on the rat caecum is very much more marked than that of equivalent quantities of inert bulking materials such as kaolin (Dowling *et al.* 1967; Sakata, 1986). A number of hypotheses have emerged to explain this difference. El Harith *et al.* (1976, 1977) suggested that the caecal enlargement in response to raw starch was an adaptive response resulting from changes in the caecal microflora. Similar conclusions were reached by Elsenhans *et al.* (1981) and Jacobs & Lupton (1984) who reported that fermentable polysaccharides gave rise to greater caecal enlargement than non-degradable polymers. These observations seem consistent with recent reports by Sakata (1986, 1987) showing that short-chain fatty acids (SCFA), which are important microbial breakdown products of carbohydrates, stimulate epithelial cell production and lead to increased mucosal growth. In contrast, however, Konishi *et al.* (1984) reported that the initial response of the rat caecum to a diet containing glucomannan was cellular hypertrophy rather than increased cell proliferation, and they suggested that the important stimulus was the physical suppression of water absorption by the hydrophilic polymer in the bowel lumen. Recently, Johnson & Gee (1986) described the growth and cytokinetics of intestinal tissues in rats fed on diets containing insoluble microcrystalline cellulose, the soluble derivative (carboxymethylcellulose; CMC), or guar gum. Despite the ready susceptibility of guar gum to fermentation by the caecal microflora (Horn *et al.* 1986), CMC gave rise to greater enlargement of the caecum and a more marked enhancement of mucosal cell proliferation than guar gum. The purpose of the present study was to explore in more detail the relation between the microbial breakdown of dietary polysaccharides and the adaptive response of both the microflora and the tissues of the large bowel in the rat.

MATERIALS AND METHODS

Animals

Male Wistar rats (130–200 g) were obtained from a commercial supplier and randomly allocated to test groups of five animals each, housed in wire-bottomed cages. All groups received feed and water *ad lib.*, and each was given the basal fibre-free diet for 5 d before changing to the test diets. The feeding period was 12 d unless otherwise indicated.

Diets

The composition of the basal fibre-free diet was (g/kg diet): starch (Snoflake maize flour; Corn Products Ltd, Manchester) 360, sucrose 300, casein (edible casein; Glaxo Farley Foods, Plymouth) 200, maize oil 80, mineral mix (Johnson *et al.* 1984) 40 and vitamin mix (Johnson *et al.* 1984) 20. In the test diets, the polysaccharides hydroxypropylmethylcellulose (HPMC; Courtaulds Chemicals & Plastics, Derby), CMC (BDH, Poole, Dorset), and carboxymethyl guar (CMG; Meyhall Chemical AG, Merseyside) replaced 100 g sucrose/ kg. Diarrhoea was induced in one group by feeding a diet in which magnesium sulphate replaced 40 g sucrose/kg.

In one experiment cellulose (SF; 'Solkafloc', Johnson Jorgensen Wettre, London) replaced 100 g sucrose/kg and guar gum (Sigma Chemical Co., Poole, Dorset) replaced 40 g sucrose/kg; both were given for 7 d.

Experimental procedure

Animals were housed in a room having a 12 h light–12 h dark cycle, and experiments were carried out in the morning. At the end of the feeding period the animals were anaesthetized by intraperitoneal injection (Euthetal, May & Baker, Dagenham; 1 ml/kg body-weight) and killed by cervical dislocation. The abdomen was opened and the caecum and ascending colon were separately ligatured, removed and weighed. The caecum was transferred to an anaerobic cabinet containing an atmosphere of nitrogen–hydrogen–carbon dioxide (85:10:5, by vol.) where it was opened, and samples of contents were reserved for weighing and subsequent drying to constant weight at 105°. A slurry was then made from a known weight (approximately 1 g) of contents in 9 ml of anaerobic dilution solution (ADS; Bryant & Burkey, 1953) for enumeration of anaerobes and aerobes on plates of complex growth media as previously described (Horn *et al.* 1986). The contents of the colon were squeezed out under a stream of O_2-free CO_2 and similarly treated. Caecal and colonic tissues were gently rinsed under running water, blotted dry and weighed, and then oven-dried to constant weight. A portion of caecal contents was frozen for subsequent analysis of SCFA by the method of Bayliss & Houston (1985).

Characterization of aerobic bacteria. All the colonies (minimum twenty) on the plates from the highest dilution were examined. Preliminary identification was made from colony morphology on the isolation medium (CLED agar; Oxoid Ltd) and confirmed by using either the AP120E system (API Laboratory Products, Basingstoke) or the methods of Cowan (1974).

Toxin testing of Escherichia coli *strains.* A Vero cell enterotoxin activity assay was used which detects both heat-labile enterotoxin and Vero cytotoxin (Scotland *et al.* 1985). Additionally, because Vero cells do not respond to heat-stable enterotoxin, serotyping of cultures was carried out.

Analysis of carbohydrate in intestinal contents of CMC-fed rats. To determine the amount of CMC present, residual starch in the ileal and colonic contents of rats fed on the CMC diet for 7 d was first removed by a modification of the method of Faulks & Timms (1985). Contents were dried over phosphorus pentoxide and ground to a fine powder; 20 mg were dispersed in 1 ml dimethyl sulphoxide and heated for 5 min at 100°. Starch was hydrolysed

by incubating this sample at 37° for 1 h with amyloglucosidase (*EC* 3 . 2 . 1 . 3; 65 units) and α-amylase (*EC* 3 . 2 . 1 . 1; 1120 units) (Sigma Chemical Co.) in 8 ml acetate buffer, pH 4·6. The remaining polysaccharide in the destarched material was precipitated by treatment with ethanol (900 ml/l final concentration). After centrifugation the precipitate was washed with ethanol (900 ml/l) and redissolved in distilled water; total carbohydrate in this solution was measured by the phenol–sulphuric acid method of Dubois *et al.* (1956). using glucose as a standard. As a measure of the extent of degradation of the CMC polymer a reducing-end assay (Dygert *et al.* 1965) was compared with a similar assay on the contents from animals fed on the fibre-free diet.

Intracaecal pH changes. In a separate experiment three groups of five rats were fed for 28 d on test-diets containing Solkafloc, CMC or guar gum at a level of 100 g/kg diet. At the end of this period the animals were killed as described above. The caecae were exposed immediately and opened at the caeco-colonic junction and the pH of the caecal contents was measured using a semi-micro pH-electrode as described by Southon *et al.* (1987).

Faecal output. The effects of the test materials on the faecal outputs of rats were assessed in a separate study. After the 12 d feeding period a complete 24 h faecal collection was made from groups of five animals as described. Where possible, discrete faecal pellets were separated from spilled food and weighed. Unformed faecal material was collected on pre-weighed paper sheets, oven-dried and re-weighed. In all cases the wet weight was determined from freshly voided material.

In vitro fermentation of polysaccharides. All media were pre-reduced and prepared using the techniques of Hungate (1969); complete anaerobiosis was maintained during subsequent manipulations by the use of the anaerobic cabinet. Utilization of guar gum, CMC, CMG, HPMC and SF was tested in the complex medium SM10 (Barnes & Impey, 1974) without glucose, cellobiose or starch (BSM10). Test substrates (2·0 mg/ml) were added aseptically to 150 ml BSM10 in 250-ml batch culture vessels using the anaerobic cabinet and dissolved or dispersed by stirring overnight at 2°. Broths were prewarmed to 37° before inoculation. Slurries of caecal contents from rats fed on the fibre-free diet were prepared in anaerobic dilution solution, as described previously. A 1·5 ml portion of slurry was used to inoculate 150 ml broth; the culture vessels were incubated at 37° using a shaking platform.

Total viable bacterial counts were estimated after 0, 6, 12, 24 and 48 h of incubation of the batch cultures. For each sample a tenfold dilution series in ADS was prepared and viable bacteria enumerated, after 7 d incubation at 37°, on plates of a complex medium (VLhlf) as described by Horn *et al.* (1986). Carbohydrate utilization was determined using the phenol-sulphuric acid method of Dubois *et al.* (1956); concentrations were read from standard curves prepared with either the polysaccharide or glucose. Samples of the batch cultures were taken after 0, 6, 12, 24, 48 h and 7 d of incubation. Free reducing-ends in the culture fluids of HPMC, CMG and CMC fermentations were determined by the method of Dygert *et al.* (1965).

Water-holding capacity (WHC) determination. The WHC of CMC, CMG, HPMC and SF was determined by the method of McBurney *et al.* (1985). An osmotic suction pressure of 89 mosmol/l (2 atm) was used for all measurements. Samples (100 mg) were first dissolved or dispersed in 5 ml sodium azide solution (1 mg/ml) to hydrate the sample.

Statistical analysis

Analysis was performed using the computerized statistical package MINITAB (Pennsylvania State University). Significance of differences between means was tested using a *t* procedure for independent samples. When required, simple regression lines or, where indicated by residual analysis, polynomial regression lines were fitted to values.

Table 1. *The effect of non-digestible dietary polysaccharides on bacterial numbers, levels of short-chain fatty acids (SCFA) and mass of caecal and colonic contents in rats*

(Values are means with their standard errors for five animals per group)

Diet* ...	Fibre free		Magnesium sulphate		HPMC		CMC		CMG	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wt of animal (g)	295	11	261	5	321	11	240	4	267	9
Caecum:										
Viable bacterial counts (log$_{10}$/g dry wt contents)										
Total anaerobic count†	11·21^a	0·05	11·49^{bd}	0·06	10·81^c	0·03	11·35^{ab}	0·07	11·71^d	0·08
Aerobic count	10·22^{ab}	0·12	10·49^a	0·12	9·82^b	0·10	11·05^c	0·08	10·20^a	0·07
Total viable bacteria per caecum‡ (log$_{10}$)	10·72^a	0·08	10·89^a	0·05	10·45^b	0·05	11·61^c	0·10	11·84^c	0·09
Total SCFA										
(μmol/g dry wt contents)	370·7^a	30·7	512·0^b	18·0	213·3^c	36·3	152·6^c	20·5	266·2^{ac}	42·0
Total SCFA per organ‡ (μmol)	82·2^a	10·9	126·0^b	11·5	94·7^{ab}	5·9	269·4^c	33·5	339·1^c	27·4
Wet wt of contents‡ (g)	1·41^a	0·21	4·03^b	0·26	3·50^b	0·38	11·24^e	1·38	6·86^d	0·93
Dry wt of contents‡ (g)	0·32^{ab}	0·04	0·25^a	0·02	0·49^b	0·07	1·85^e	0·25	1·42^e	0·26
Moisture in contents (g/kg)	765^a	17	939^b	3	862^c	7	837^d	3	796^{ad}	15
Wt of caecal tissue‡ (g)	0·60^a	0·04	1·13^{bd}	0·05	0·96^b	0·06	1·44^c	0·10	1·24^{cd}	0·05
Colon:										
Viable bacterial counts (log$_{10}$/g dry wt contents)										
Total anaerobic count†	11·20^{ad}	0·06§	11·59^b	0·06§	10·83^c	0·11	11·32^d	0·07	11·89^{ac}	0·22§
Aerobic count	10·21^a	0·18§	10·50^a	0·16§	9·91^a	0·10	11·25^b	0·06	10·46^a	0·12§
Total viable bacteria per colon‡ (log$_{10}$)	10·20^a	0·12	10·17^a	0·04	10·07^a	0·11	10·74^b	0·06	11·09^{ah}	0·21§
Wet wt of contents‡ (g)	0·38^a	0·08§	0·53^a	0·08	1·55^b	0·05	1·50^b	0·17	0·62^a	0·10
Dry wt of contents‡ (g)	0·09^a	0·02§	0·04^a	0·01§	0·17^b	0·01	0·27^c	0·03	0·16^b	0·00§
Moisture in contents (g/kg)	744^a	21§	929^b	3§	886^c	3	815^d	6	765^a	9§
Wt of colonic tissue‡ (g)	0·61^a	0·03	0·80^b	0·03	1·08^c	0·09	1·05^c	0·07	0·71^{ab}	0·06

HPMC, hydroxypropylmethylcellulose; CMC, carboxymethylcellulose; CMG, carboxymethyl guar.

[a,b,c,d] Mean values in the same horizontal line with unlike superscript letters are significantly different (Student's *t* test): *P* = 0·05.

* For details, see p. 198.

† Includes facultatively anaerobic bacteria.

‡ Relative to 250 g body-weight (value × 250 g/actual body-weight).

§ Values for four animals.

Table 2. *The effect of non-digestible polysaccharides on wet weight and moisture content of faeces in rats*

(Values are means with their standard errors for five animals per group)

Diet*	Faecal output (g/24h)				Moisture content (g/kg)
	Wet		Dry		
	Mean	SE	Mean	SE	
FF	1·23	0·13	0·79	0·06	355
Cellulose	5·22	0·40	3·81	0·12	275
CMC	13·78	2·43	3·46	0·60	747
HPMC	11·59	0·99	3·43	0·14	702
Guar gum	1·53	0·35	0·81	0·13	457
CMG	7·13	0·71	2·96	0·29	588
Magnesium sulphate	17·08	4·25	2·58	0·50	841

FF, fibre free; CMC, carboxymethylcellulose; HPMC, hydroxypropylmethylcellulose; CMG, carboxymethyl guar.
* For details, see p. 198.

RESULTS

Large-bowel morphology and contents

The effects of HPMC, CMC and CMG on the weight, contents and microflora of the caecum and colon are compared with those of rats on a fibre-free diet in Table 1. The effect of a MgSO$_4$-supplemented diet is also shown. Differences in the daily faecal output of animals given the test and control diets are shown in Table 2.

All the supplemented diets led to a visually obvious enlargement of the caecum, which was associated with increased wet weight of contents, and of tissue. As previously observed (Johnson & Gee, 1986), the enlargement was particularly marked in the CMC-supplemented group, which had an almost eightfold increase in weight of caecal contents coupled with a doubling of tissue weight. The polysaccharides also significantly increased the dry weight of caecal contents. Similar increases in the colon contents and tissue weight occurred, but these were not always statistically significant.

Fig. 1 illustrates the relation between wet weight of caecal contents, and tissue mass in experiments in which the feeding period and polysaccharide supplement were varied. There was a strong correlation in both the caecum (r 0·93) and colon (r 0·94). No significant correlations were found between tissue weight and bacterial density, total number of bacteria per organ, moisture content, or SCFA concentration.

The animals fed on CMC- and MgSO$_4$-containing diets developed diarrhoea (profuse unformed stools) from the 2nd day of feeding, and the total output of faecal material was significantly increased (Table 2). The highest output of wet faecal material occurred in the MgSO$_4$-fed rats, which also had the highest moisture content in caecal and colonic contents, and in the faecal material.

Caeco-colonic microflora

The density of bacteria in both the caecum and colon was increased by all the dietary treatments except HPMC and CMC; HPMC was associated with a significant reduction compared with the fibre-free controls (Table 1). The increased caecal mass in the animals fed on CMC and CMG led to their having considerably greater total bacterial numbers per caecum than the fibre-free controls. A markedly higher proportion of aerobic bacteria was
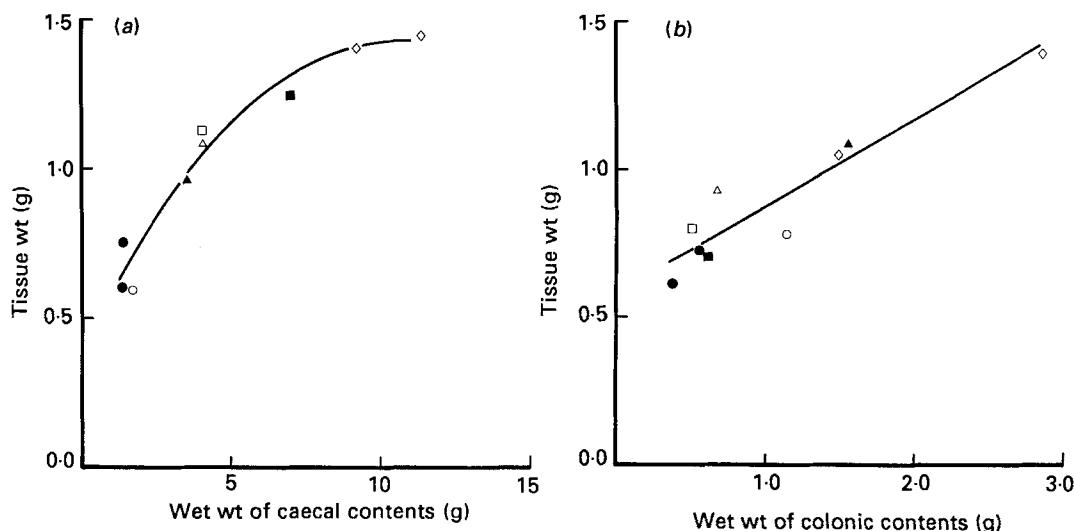
Fig. 1. The effect of weight of contents on (a) caecal and (b) colonic tissue weight in rats fed on diets containing non-digestible polysaccharides for 12 d. Diets were fibre-free (●), hydroxypropylmethyl-cellulose (▲), MgSO$_4$ (□), carboxymethyl guar (■) and carboxymethylcellulose (◇); for details see p. 198. Each point is the mean of five animals. Additional points for fibre-free (●), guar gum (△), carboxymethylcellulose (◇) and Solkafloc (○) diets given for 7 d have been added to the 12 d feeding period values. Regression lines are fitted to the values:

Caecum

$$y = 0.392 + 0.20x - 0.0097x^2 \ (r\ 0.93),$$

Colon

$$y = 0.576 + 0.296x \ (r\ 0.94).$$

Table 3. *Analysis of carbohydrate in intestinal contents of rats fed on fibre-free or carboxymethylcellulose- (CMC) supplemented diets**

(Values are means with their standard errors for five animals per group)

	Ileum		Colon	
	Mean	SE	Mean	SE
CMC in contents (mg/20 mg dry contents)				
CMC diet	6·63	2·75	8·92	0·23
Free reducing ends in contents (mg/20 mg dry contents†)				
Fibre-free diet	0·66	0·14	0·58	0·09
CMC diet	0·54	0·10	0·54	0·06

* For details, see p. 198.
† Glucose equivalents.

found in both caecal and colonic contents of rats fed on CMC compared with other diets, and these were almost entirely *E. coli* (93 % of isolates). In contrast, the aerobic flora of the fibre-free group was composed primarily of *Streptococcus* spp. (95–100 % of isolates). No enterotoxin activity was detected in *E. coli* strains isolated from CMC-fed rats with diarrhoea; serotyping showed the strains to be in groups O 54 and O 24 which are not serogroups which commonly cause diarrhoea in man (Gross, 1983).
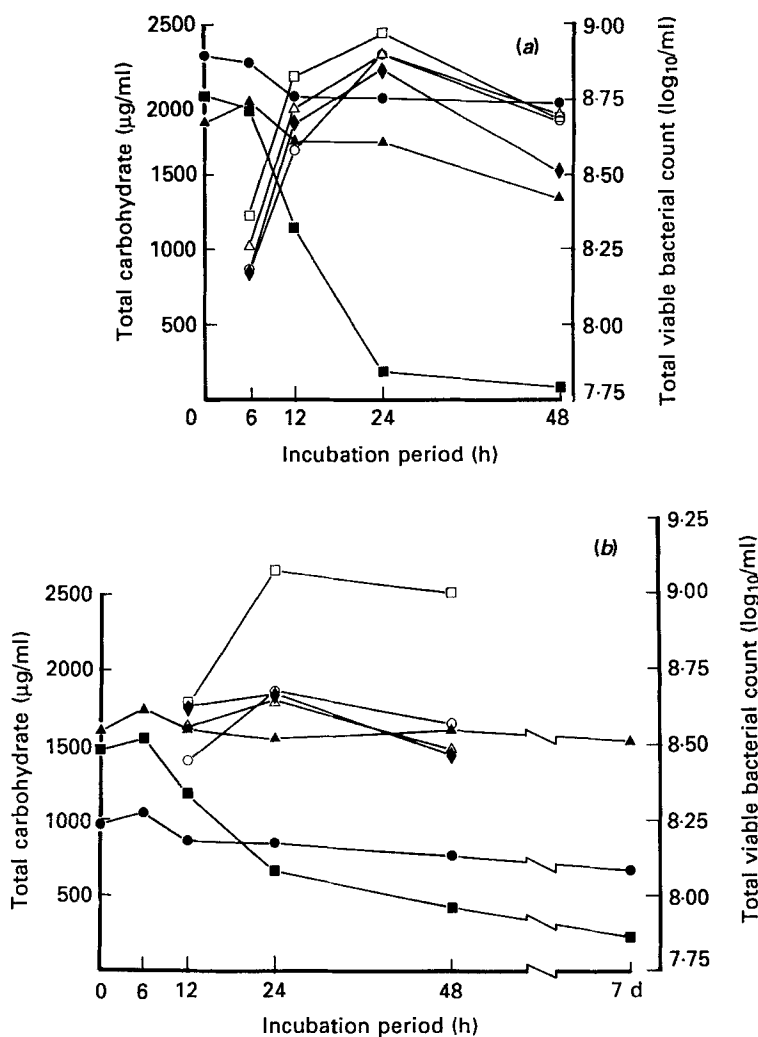
Fig. 2. Carbohydrate utilization (closed symbols) and bacterial growth (open symbols) in vitro. (*a*) BSM10 broth alone (◆); BSM10 broth with (2·0 mg/ml) guar gum (■), carboxymethylcellulose (●) or Solkafloc (▲); values are means for four broths. (*b*) BSM10 broth alone (◆); BSM10 broth with (2·0 mg/ml) carboxymethyl guar (■), carboxymethylcellulose (●) or hydroxypropylmethylcellulose (▲); values are means for two broths. The values for total carbohydrate have been corrected for basal medium values.

Intralumen SCFA levels

The highest caecal concentration of total SCFA was found in animals fed on the $MgSO_4$ diet and these also had the lowest caecal dry weight content. The lowest SCFA concentrations occurred in those fed on CMC and HPMC diets. In contrast, the highest total per organ was found with the CMC and CMG diets, reflecting the higher weight of caecal contents in these animals (Table 1).

Intralumen degradation of CMC

A substantial amount of unfermented CMC was present in the intestinal contents of the rats fed on the CMC diet, and the proportion of CMC increased distally. The CMC

Table 4. *Measurement of free reducing-ends* in fluid from caecal bacterial cultures containing hydroxypropylmethylcellulose (HPMC), carboxymethyl guar (CMG) or carboxymethylcellulose (CMC)*

Incubation period (h)	Substrate		
	HPMC	CMG	CMC
0	210	170	0
6	150	140	0
12	160	270	0
24	140	250	0
48	220	280	0
7 d	150	230	50

* μg/ml glucose equivalents, values adjusted for basal medium control.

appeared not to have been hydrolysed to shorter chain lengths, as no increase in reducing ends could be demonstrated compared with the fibre-free diet, and there was no change in reducing-end concentration down the length of the intestine (Table 3).

Intracaecal pH

The mean pH of the caecal contents of rats fed on CMC (6·65, SE 0·10) did not differ significantly from that of rats fed on SF (6·75, SE 0·08). In contrast the caecal contents of rats given guar gum (pH 5·91, SE 0·13) were significantly more acidic than both the CMC- and SF-fed groups ($P < 0.01$).

In vitro degradation of the polysaccharides

Fermentation of guar gum was rapid, with 95% of the polysaccharide being utilized after 24 h incubation at 37° (Fig. 2(*a*)). Utilization of CMG was slower, with 28% remaining after 48 h incubation. Further incubation reduced this to 15% (Fig. 2(*b*)). CMC and SF were poorly fermented even after prolonged incubation, and HPMC remained almost completely unfermented, with only 5% of the substrate having been utilized after 7 d of incubation (Fig. 2). By 7 d of incubation there was only a very slight increase in the level of reducing-ends in the culture fluid with CMC as the substrate, but no significant change in reducing-ends for HPMC was recorded (Table 4); thus there appeared to be little or no degradation of these cellulose polymers. There was apparently a slight accumulation of reducing-ends with CMG as substrate and the final value was similar to the total measurable carbohydrate (Fig. 2(*b*)), suggesting that the carbohydrate residue after fermentation may have been composed of non-utilizable methylated monomers.

The total viable bacterial count of the broths immediately after inoculation was ($\log_{10}$/ml) 6·05 (Fig. 2 (*a*)) or 7·09 (Fig. 2(*b*)). Viable bacterial counts in the cultures after 24 h incubation were highest for guar gum and CMG (Fig. 2). Counts in SF cultures were slightly above those of the basal medium (Fig. 2(*a*)) and by 24 h CMC counts had also increased slightly above the basal value (Fig. 2). HPMC counts did not rise above those of the basal medium (Fig. 2(*b*)).

WHC

The WHC (g water/g organic matter) of the different polysaccharides was as follows (values are the means of duplicate analyses): CMC 12·8 (SE 0·3); CMG 10·2 (SE 0·8); HPMC 6·6 (SE 1·8); SF 2·1 (SE 0·8). These results do not support the findings of McBurney *et al.* (1985) that fibres with the highest WHC were also the most fermentable.

DISCUSSION

In the present study, the addition of non-digestible polysaccharides to a basal, fibre-free rat diet led to a considerable enlargement of the caecum and colon. As in earlier experiments, the effect was most marked in the case of CMC, a material which we have previously shown to be associated with a particularly high rate of mucosal cell proliferation in the mid- and distal small intestine (Johnson & Gee, 1986).

In several recent studies by other authors it has been reported that fermentable polysaccharides cause marked caecal enlargement associated with an increase in mucosal cell proliferation, and it has been argued that this is due to increased bacterial metabolism leading to a reduction in intralumen pH (Lupton *et al.* 1985), or a direct stimulatory effect of SCFA which Sakata (1987) regards as specific lumen trophic factors. This hypothesis is not supported by the present study since CMC was not significantly degraded during passage through the large bowel nor during prolonged incubation with faecal microflora in vitro, nor did it increase the density of bacteria in either the caecal or colonic contents. The pH of caecal contents for rats fed on CMC, compared with the highly fermentable guar gum, confirms that CMC was not fermented in vivo. The concentration of SCFA was lowest in the CMC-fed animals, although the enlargement of their caeca and the retention of solids and fluid led to their having the highest total load of SCFA per organ. Conning *et al.* (1983) reported an increase in the activity per caecum of several enzymes in rats fed on CMC, and concluded that the $\beta1 \rightarrow 4$-glucose backbone of CMC was readily assimilated by the gut flora. It would appear, however, that the increase in β-glucosidase activity per caecum could be accounted for by the increase in contents, which they also found.

When the mass of caecal contents was varied by altering the type, dietary level and duration of intake of dietary polysaccharides, there was a strong correlation between wet weight of caecal contents and tissue weight, suggesting that caecal and colonic enlargement may be a simple adaptive response to increased bulk of contents, whatever their nature. Such a mechanism may also account for the enlarged caeca found in germ-free rats where undigested mucus and food residues are known to accumulate.

Rather surprisingly, the concentration of SCFA in caecal contents was highest for the $MgSO_4$ diet, partly perhaps because of the low retention of dry material in the caeca of these animals. SCFA concentrations in general did not parallel the density of bacteria, confirming our previous view that bacterial metabolism, or the rate at which SCFA are absorbed, may be modified by dietary consitutents other than fermentable polysaccharides (Horn *et al.* 1986).

Diarrhoea in rats fed on non-digestible polysaccharides appears not to be linked entirely to the moisture content of the faeces; CMC and HPMC diets gave faeces with similar moisture content, yet the stools of CMC-fed animals were poorly formed whilst those of the HPMC-fed animals were bulky, well formed pellets. Similar results were found with CMC and sodium alginate (Gohl & Gohl, 1977), and with hydroxypropyl distarch phosphate (HDP) and tapioca starch (Wang, 1979). In each case both polysaccharides led to a high moisture content in the faeces or in the caecal contents, but only CMC or HDP caused diarrhoea. Diarrhoea did not occur in human volunteers consuming 15 g CMC/d, which would presumably have been about 2–3 % of their intake of dry matter (Anderson *et al.* 1986). The reason for the onset of diarrhoea in CMC-fed animals in the present study remains unclear. It may reflect the form of bound water and the physical or chemical properties of the polysaccharide in the faeces or it may have resulted from differences in motility. These possibilities are not of course mutually exclusive.

The bacterial populations of the caecum and colon varied significantly with diet. CMG gave the largest population in the present study, as did guar gum in our previous work

(Horn *et al.* 1986). HPMC, like insoluble microcrystalline cellulose in our previous study, led to a reduced density of bacteria in caecal contents compared with that of animals given the fibre-free diet. This was not due to a diluting effect of undigested polysaccharide (Mallet *et al.* 1983), as the dry weight of contents was not increased and therefore the total number of bacteria in the caecum was also significantly lower on these diets.

The differences between the aerobic bacterial populations on the different diets were notable, but the significance of this finding is not known. Bruns *et al.* (1977) also found marked differences in the caecal aerobic flora with diet, with higher counts of lactobacilli and lower counts of streptococci on diets containing unmodified starch compared with diets containing hydroxypropyl starch. Gorbach (1971) pointed out that reductions in faecal anaerobes can occur as a consequence of diarrhoea. The animals given a $MgSO_4$-supplemented diet also developed diarrhoea of similar severity to that seen in the rats given CMC, but there was no significant increase in the proportion of aerobes in these animals, nor in those given CMG, another carboxymethylated compound. The high proportion of aerobes, which proved to be almost entirely *E. coli*, in both the caecum and colon of the CMC-fed rats raised the possibility that the diarrhoea could have been due to enterotoxigenic strains of this organism, but we obtained no evidence to support this hypothesis.

The present study establishes that the enlargement of the rat caecum in response to non-digestible polysaccharides can occur in situations where the polysaccharide is resistant to fermentation and therefore does not provide a substrate for bacterial metabolism. The adaptive response is not therefore primarily a response to bacterial fermentation. Hence, despite the striking ability of SCFA to stimulate mucosal cell proliferation (Sakata, 1987), the importance of this effect as a hypertrophic stimulus causing enlargement of gastrointestinal organs is doubtful. A possible alternative explanation is that the caecum enlarges to accommodate the tendency of residual material to accumulate within it, and this is governed by the pattern of motility and bulk flow through the whole large bowel. This, in turn, may depend primarily on the physical properties of the undigested residue, such as water retention and viscosity, both of which are relatively high in the modified cellulose gums. Even so there were important differences in the physical forms of stools produced by the two modified cellulose gums in the present study, and the reasons for this remain unclear. It is important that these effects should be explored in man, since modified cellulose gums are widely used as bulk laxatives and apparently minor differences in their chemical structure may have important clinical implications.

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