Effects of galacto-oligosaccharide and bacterial status on mucin distribution in mucosa and on large intestine fermentation in rats

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The purpose of the present paper was to study the effects of a dietary undigestible carbohydrate and intestinal microflora on mucin distribution (neutral, acid, sulphonated), glycolytic activities: β-D-galactosidase (*EC* 3.2.1.23), *N*-acetyl-β-D-galactosaminidase (*EC* 3.2.1.43), *N*-acetyl-β-D-glucosaminidase (*EC* 3.2.1.30), α-L-fucosidase (*EC* 3.2.1.51) and bacterial metabolism (gas production, short-chain fatty acids (SCFA) and lactic acid caecal concentration) in germ-free (GF), conventional (CV) and heteroxenic (HE) rats (GF rats associated with a human flora). Rats were fed on either a control diet or a diet containing 40 g *trans*-galactosylated oligosaccharide (TOS)/kg. In GF rats fed on the control diet caecal pH was almost neutral and glycolytic activities negligible. The number of mucus-containing cells increased from the caecum to the colon for the three types of mucin. TOS had no effect in the caecum but it modified mucin cell repartition in the colon. In CV and HE rats fed on the control diet caecal pH was similar (6·8), but caecal SCFA and lactic acid concentrations (μmol/g) and gas production (ml/24 h) were higher in CV (70, 5·9 and 2·3 respectively) than in HE rats (32, 4·6 and 0·4 respectively). In CV, as in HE rats, acid-mucin-containing cells increased from the caecum to the colon and glycolytic activities were similar. TOS reduced acid-mucin-containing cells in the caecum of CV rats by twofold but had no effect in either the caecum or the colon of HE rats. TOS strongly increased β-galactosidase activity and slightly modified the other glycolytic activities. Its effect on bacterial metabolites depended on bacterial status. However, comparison between CV and HE rats showed no evident relationship between the number of mucus-containing cells and measured bacterial metabolites. Differences between CV and HE rats might be due to bacterial microflora specificity. TOS had an intrinsic effect on mucus cell distribution in the colon of GF rats. In CV and HE rats the presence of the flora abolished this effect.

Bacteria: Hindgut: Mucins: Oligosaccharide

Dietary fibres have complex effects on intestinal mucosa modifications (Cassidy *et al.* 1981); they induce activities of glycolytic enzymes which could participate in mucin degradation (Salyers et al. 1977; Hoskins & Boulding, 1981).

Wheat bran and, to a lesser extent, cellulose increase labelled sulphate and [^{3}H]glucose incorporation into intestinal glycoproteins and mucins in rats (Schneeman *et al.* 1982; Vahouny *et al.* 1985).

Bran, pectin or gum increase acid mucin in the colon of pigs with less of neutral mucin (Moré *et al.* 1987).

The effects of natural fibres may be attributed either to their mechanical properties or to the endproducts of their fermentation by bacteria, i.e. the short-chain fatty acids (SCFA).

Bacterial flora alters mucin secretion; for example, compared with germ-free (GF) animals conventional (CV) animals exhibit fewer goblet cells in the large intestine of

guinea-pigs (Sprinz *et al.* 1961) and piglets (Staley *et al.* 1970), mid small intestine and colon of piglets (Heneghan *et al.* 1979) and small intestine of dogs (Heneghan, 1979). GF rats and mice accumulate more mucus in their caecal contents (Loesche, 1968; Combe *et al.* 1976). However, conventionalization of GF rats results in hyperplasia of the crypt epithelial cells, including mucus-producing cells in the ileum and caecum (Ishikawa *et al.* 1986, 1989).

A *trans*-galactosylated oligosaccharide (TOS) is actually used in Japan in human food as a bifidobacterial factor (Ito *et al.* 1990). In humans TOS seems to be poorly digested by endogenous enzymes and is fermented by intestinal flora, as shown by breath test studies (Tanaka *et al.* 1983).

The purpose of the present paper was to study the effects of TOS on mucin (neutral, acidic, and sulphonated) distribution in the mucosa, bacterial glycolytic activities and bacterial fermentations. In order to dissociate the effects of TOS itself from those of different types of flora, GF and CV rats and rats born GF and inoculated with a human flora (heteroxenic (HE) rats) were compared, since the origin of the bacterial flora may lead to different fermentation products.

MATERIALS AND METHODS

Animals

Male GF, CV and HE inbred F344 rats (3 months old) were used. HE rats were obtained by inoculation of GF rats with human faecal flora. Human faeces provided by a methano-producer were diluted 1:20 (w/v) with NaCl (9 g/l) in an anaerobic chamber; 1 ml of this dilution was inoculated orally twice during 24 h. Temperature and relative humidity of the animal room were controlled ($21 \pm 2°$, $60 \pm 5\%$ respectively). The lighting schedule was also controlled (12 h light–12 h dark). Rats were placed in wire-mesh cages and GF and HE rats were kept in isolators (La Calhene, France) as already described (Le Coz *et al.* 1989).

Diets

Two diets were used; a control diet containing (g/kg) maize starch 660, maize oil 40, fish meal 230, kaolin 50, minerals and vitamins mixture (compositions previously described by Andrieux & Sacquet (1986)) 20, and an experimental diet in which 40 g maize starch/kg was replaced by 40 g TOS/kg. Sterilized feed (gamma irradiation, 40 kGy in vacuum-sealed plastic bags) and water were given *ad lib.* to the rats.

Experimental design

Groups of four GF, CV and HE rats were fed for 1 month on the control or experimental diets. At the end of this adaptation period the H_2 and CH_4 produced were collected in a respiratory chamber (Le Coz *et al.* 1989). Then rats were killed by an overdose of sodium pentobarbitone (SANOFI, Aulnay/Bois, France; 60 mg/kg intraperitoneally). Caecal pH was measured. Intestinal segments with contents were taken from the caecum, proximal colon (20 mm aboral to the caecum) and distal colon (20 mm oral to the rectum), frozen in isopentane cooled with liquid N_2 and stored at $-80°$ until required for sectioning.

The remaining caecal contents were frozen in liquid N_2 and maintained at $-20°$ until required for determination of SCFA, D- and L-lactic acids and bacterial glycolytic activities.

Histological techniques

Sections (10 μm thick) were cut at $-20°$ with a cryostat (Reichert-Jung Frigocut 2700) and fixed with formaldehyde vapour at 40° overnight. Sections were stained with periodic acid–Schiff reaction (PAS) for neutral mucin (McManus, 1946), alcian blue 8 GX at pH 2·5

(AB 2·5) for acid mucin (Mowry & Winkler, 1956) and by high-Fe–diamine reaction without previous oxidation (HID) for sulphomucins (Spicer, 1965) and with a slight modification in the staining solution (400 g $FeCl_3$/l instead of 100 g $FeCl_3$/l).

For each stain, histological slides were number-coded and examined for quantification by two observers unaware of the origin of the slides.

Measurements of mucus-containing cells

In the caecum, proximal and distal colon, on sections perpendicular to the mucosa, the number of mucus-containing cells in the crypt, cut from the base to the neck, was counted for twenty crypts on each specimen. The stain was taken up by the luminal mucus layer adherent to the epithelial cells and by the intraluminal mucus dispersed in the contents of the caecum only in CV and GF rats, and the extent of staining was expressed on a relative scale (±, null or faint; +, weak; + +, moderate; + + +, intense).

Analytical procedure

H_2 and CH_4 were measured using a Quintron apparatus (model DP; Quintron-Instrument Co, Milwaukee, USA), SCFA by GLC (Szylit *et al.* 1988). D- and L-lactic acids were determined enzymically (UV Boehringer method; Meylan, France). Glycosidase activities were measured by the rate of release of *p*-nitrophenol from their *p*-nitrophenylglycoside. The reaction mixture contained 0·3 ml substrate solution (0·5 mM) and 0·2 ml caecal contents in phosphate buffer (0·1 M) at the pH of the caecum. Incubation was performed at 37° for 5, 10, and 30 min and *p*-nitrophenol concentration was measured as the absorbance at 400 nm after adding 2·5 ml 0·25 M-Na_2CO_3. Activities were expressed as U/g wet caecal content. One unit was equivalent to 1 μmol *p*-nitrophenol released/min.

Statistical analysis

The results are expressed as means with their standard errors. Experimental values were compared by two-way analysis of variance with subsequent Fisher PLSD, Scheffé *F* and Dunnett *T* tests and with the Newman–Keuls multiple-range test (STATITCF software; ITCF, Paris, France).

RESULTS

Mucus-containing cells in caecum, proximal and distal colon

The caecal mucosa (Table 1) of GF rats fed on the control diet exhibited less mucus-containing cells than that of CV rats irrespective of the mucin type studied. In GF rats, as in CV rats, the number of AB 2·5-positive cells was lower than that of PAS- and HID-positive cells; tenfold in GF and threefold–fourfold in CV rats. Compared with their CV counterparts, HE rats had similar numbers of PAS- and HID-positive cells and significantly more AB 2·5-positive cells.

TOS had no effect in GF rats and very slight effects in CV and HE rats; there were fewer AB 2·5-positive cells in CV and more HID-positive cells in HE rats.

There was a greater extent of staining with AB 2·5 of the subepithelial lumen mucus layer in close contact to the epithelium in GF rats fed both with or without TOS in the diet when compared with CV rats (Table 2). Secreted mucus, assessed by the extent of staining, was more abundant in the intraluminal caecal contents than in the subepithelial lumen mucus layer, both for GF and CV rats, despite slight differences between mucin types.

In the proximal and the distal colon differences between GF and HE rats were small (Table 3). In the proximal colon of GF rats fed on the control diet the number of PAS-positive cells was higher than that in CV rats, and HE rats had more PAS-positive and less AB 2·5-positive cells than CV rats.

Table 1. *Number of mucus-containing cells per crypt section in the caecum of germ-free (GF), conventional (CV) and heteroxenic (HE) rats fed on control (C) or* trans-*galacto-oligosaccharide (TOS) diet**

(Mean values for four rats with their pooled standard errors; error df 18)

Rats...	GF		CV		HE			Variance analysis		
Diet...	C	TOS	C	TOS	C	TOS	Pooled SEM	Flora	Diet	Inter-action
Mucin type										
Neutral (PAS+)	10^{b}	9^{b}	20^{a}	18^{a}	21^{a}	22^{a}	0·39	0·001	NS	NS
Acid (AB 2·5+)	1^{d}	1^{d}	5^{b}	$2·5^{c}$	$8·2^{a}$	$8·5^{a}$	0·20	0·001	0·05	0·05
Sulpho (HID+)	9^{d}	10^{d}	16^{c}	14^{c}	$17·7^{bc}$	$19·8^{a}$	0·33	0·001	NS	NS

[a, b, c, d] Mean values within the same horizontal line with unlike superscript letters were significantly different (Newman–Keuls test): $P < 0·05$.

PAS, periodic acid–Schiff; AB 2·5, Alcian blue pH 2·5; HID, high-Fe–diamine; +, positive response to stain; NS, not significant.

* For details of animals and diets, see p. 904.

Table 2. *Extent of staining** *of subepithelial luminal mucus layer and intraluminal mucus contents in the caecum of germ-free (GF) and conventional (CV) rats fed on control (C) or* trans-*galacto-oligosaccharide (TOS) diet*†

(Mean values for four rats with their pooled standard errors)

Rats...	GF		CV	
Diet...	C	TOS	C	TOS
Subepithelial luminal mucus layer				
Mucin type				
Neutral (PAS+)	+	++	+	++
Acid (AB 2·5+)	++	++	+	+
Sulpho (HID+)	++	++	++	+/−
Intraluminal mucus contents				
Mucin type				
Neutral (PAS+)	++	++	++	+++
Acid (AB 2·5+)	+++	+++	+++	++
Sulpho (HID+)	++	++	++	+++

PAS, periodic acid–Schiff; AB 2·5, Alcian blue pH 2·5; HID, high-Fe–diamine.

* Relative scale of intensity of staining; +/−, null or faint; +, weak; ++, moderate; +++, intense.

† For details of animals and procedures, see pp. 904–905.

In the distal colon GF and HE rats had more PAS-positive cells and less AB 2·5- and HID-positive cells than CV rats.

With TOS in GF rats there were significantly less PAS, AB 2·5- and HID-positive cells in the proximal colon mucosa and more PAS-, AB 2·5- and HID-positive cells in the distal colon. CV rats had a significant increase in AB 2·5-positive cells, with no change in the other mucin-containing cells in the proximal colon and only a slight decrease of HID-positive cells in the distal colon.

In HE rats slight variations occurred in PAS-, AB 2·5- and HID-positive cells with TOS;

Table 3. *Number of mucus-containing cells per crypt section in the proximal and distal colon of germ-free (GF), conventional (CV) and heteroxenic (HE) rats fed on control (C) or* trans-*galacto-oligosaccharide (TOS) diet**

(Mean values for four rats with their pooled standard errors; error df 18)

Rats...	GF		CV		HE			Variance analysis		
Diets...	C	TOS	C	TOS	C	TOS	Pooled SEM	Flora	Diet	Interaction
Proximal colon										
Mucin type										
Neutral (PAS+)	34^{a}	13^{e}	22^{d}	23^{d}	29^{c}	$27{\cdot}2^{b}$	0·40	0·05	0·001	0·001
Acid (AB 2·5+)	17^{c}	12^{d}	21^{b}	27^{a}	$16{\cdot}7^{c}$	19^{bc}	0·40	0·001	NS	0·001
Sulpho (HID+)	21^{bc}	$12{\cdot}5^{a}$	19^{b}	21^{bc}	$21{\cdot}3^{bc}$	$18{\cdot}1^{b}$	0·41	0·002	0·005	0·001
Distal colon										
Mucin type										
Neutral (PAS+)	18^{c}	27^{a}	$16{\cdot}5^{d}$	16^{d}	22^{b}	$18{\cdot}4^{c}$	0·38	0·001	0·001	0·001
Acid (AB 2·5+)	19^{c}	24^{a}	21^{b}	22^{b}	14^{d}	$14{\cdot}2^{d}$	0·53	NS	NS	NS
Sulpho (HID+)	16^{d}	$23{\cdot}5^{b}$	22^{b}	$19{\cdot}5^{c}$	$17{\cdot}3^{d}$	20^{c}	0·32	NS	0·002	0·001

[a, b, c, d, e] Mean values within the same horizontal line with different superscript letters were significantly different (Newman–Keuls test): $P < 0{\cdot}05$.
PAS, periodic acid–Schiff; AB 2·5, Alcian blue pH 2·5; HID, high-Fe–diamine; +, positive response to stain; NS, not significant.
* For details of diets and procedures, see pp. 904–905

Table 4. *Glycosidase activities (U/g wet caecal content) of germ-free (GF), conventional (CV) and heteroxenic (HE) rats fed on control (C) or* trans-*galacto-oligosaccharide (TOS) diet**

(Mean values for four rats with their pooled standard errors; error df 18)

Rats...	GF		CV		HE			Variance analysis		
Diet...	C	TOS	C	TOS	C	TOS	Pooled SEM	Flora	Diet	Interaction
β-D-galactosidase (*EC* 3.2.1.23)	$0{\cdot}015^{d}$	$0{\cdot}015^{d}$	$1{\cdot}0^{c}$	$14{\cdot}6^{a}$	$1{\cdot}4^{c}$	$9{\cdot}0^{b}$	0·2	0·001	0·001	0·001
N-acetyl-β-D-galactosaminidase (*EC* 3.2.1.53)	nd	nd	$0{\cdot}88^{a}$	$0{\cdot}62^{b}$	$0{\cdot}76^{ab}$	$0{\cdot}85^{a}$	0·05	0·001	NS	0·05
N-acetyl-β-D-glucosaminidase (*EC* 3.2.1.30)	nd	nd	$3{\cdot}4^{a}$	$1{\cdot}7^{b}$	$1{\cdot}7^{b}$	$2{\cdot}3^{b}$	0·4	0·001	0·05	0·001
α-L-Fucosidase (*EC* 3.2.1.51)	nd	nd	$0{\cdot}08^{b}$	$0{\cdot}09^{b}$	$0{\cdot}06^{b}$	$0{\cdot}28^{a}$	0·01	0·001	0·001	0·001

[a, b, c, d] Mean values within the same horizontal line with different superscript letters were significantly different (Newman–Keuls test): $P < 0{\cdot}05$.
nd, not detected.
* For details of diets and procedures, see pp. 904–905

Table 5. *Gas production (ml/24 h) of germ-free (GF), conventional (CV) and heteroxenic (HE) rats fed on control (C) or* trans*-galacto-oligosaccharide (TOS) diet**

(Mean values for four rats with their pooled standard errors; error df 18)

Rats...	GF		CV		HE			Variance analysis		
Diet...	C	TOS	C	TOS	C	TOS	Pooled SEM	Flora	Diet	Inter-action
H_2	nd	nd	0·35[b]	0·76[b]	0·19[b]	1·63[a]	0·25	0·01	0·01	0·05
CH_4	nd	nd	2·06[b]	3·41[b]	0·18[c]	5·02[a]	0·42	0·001	0·001	0·001

[a, b, c] Mean values within the same horizontal line with different superscript letters were significantly different (Newman–Keuls test): $P < 0·05$.

nd, not detected.

* For details of diets and procedures, see pp. 904–905.

a decrease in PAS-positive cells in the proximal colon, with a decrease in PAS-positive cells and an increase in HID-positive cells in the distal colon.

Glycosidase activities in caecal content

In GF rats a very low β-galactosidase (*EC* 3.2.1.23) activity was observed whatever the diet (Table 4). CV and HE rats fed on control diets exhibited only small differences in the *N*-acetyl-β-D-glucosaminidase (*EC* 3.2.1.30) activity which was lower in HE rats than in CV rats. The major effect of TOS intake in CV and HE rats was an increase in the β-galactosidase activity; however, this effect was more pronounced in CV rats. The other enzymes studied were only slightly modified: TOS lowered *N*-acetyl-β-D-galactosaminidase (*EC* 3.2.1.53) and *N*-acetyl-β-D-glucosaminidase in CV rats and increased α-L-fucosidase (*EC* 3.2.1.51) in HE rats.

Gas production

There was no H_2 and CH_4 production in GF rats (Table 5). With CV and HE rats fed on the control diet, H_2 production was similar (about 0·2 ml/24 h) but CH_4 production differed greatly (2 ml/24 h for CV rats *v.* 0·18 ml/24 h for HE rats).

TOS intake increased significantly H_2 and CH_4 production in HE rats and tended to increase, but not significantly, H_2 and CH_4 production in CV rats (interaction flora × diet $P < 0·001$).

Caecal pH, SCFA and lactic acid concentration

GF rats were characterized by an approximately neutral caecal pH (6·8–6·9), a low SCFA caecal concentration (only acetate) and no lactic acid, independently of the diet (Table 6).

In all rats fed on the control diet caecal pH was similar. L-lactic acid concentration was similar in CV and HE rats but D-lactic acid was threefold lower in HE rats than in CV rats. SCFA concentration was higher in CV rats than in HE rats and the composition was different: the caecum of CV rats contained less acetate and more propionate, valerate and the corresponding iso-acids than that of HE rats.

TOS intake lowered the pH but this effect was higher in CV rats than in HE rats (interaction $P < 0·001$). TOS slightly increased L-lactic acid and decreased D-lactic acid (3·8–1·5 μmol/g) in CV rats; TOS decreased L-lactic acid in HE rats. TOS did not increase SCFA caecal concentration significantly, but it slightly increased butyrate proportion in the caecum of CV and HE rats, propionate proportion in CV rats and proportion of valerate in HE rats. It significantly decreased isovalerate and isobutyrate in CV and HE rats.

Table 6. *Caecal pH, SCFA and lactic acid of germ-free (GF), conventional (CV) and heteroxenic (HE) rats fed on control (C) or* trans*-galacto-oligosaccharide (TOS) diet**

(Mean values for four rats with their pooled standard errors; error df 18)

Rats...	GF		CV		HE		Pooled SEM	Variance analysis		
Diet...	C	TOS	C	TOS	C	TOS		Flora	Diet	Inter-action
pH	6·8[a]	6·9[a]	6·8[a]	6·2[c]	6·8[a]	6·6[b]	0·03	0·001	0·001	0·001
L-lactic acid (μmol/g)	nd	nd	2·1[a]	3·6[bc]	2·8[c]	1·9[ab]	0·6	0·001	NS	0·05
D-lactic acid (μmol/g)	nd	nd	3·8[b]	1·5[a]	1·8[ab]	1·3[b]	0·5	0·001	0·001	0·001
SCFA (μmol/g)	0·1[c]	0·1[c]	71[c]	83[a]	32[b]	43[b]	8	0·01	NS	NS
C2%	100	100	64[c]	60[c]	72[b]	73[b]	2	0·001	NS	NS
C3%	—	—	22[b]	28[a]	18[c]	15[c]	1	0·001	NS	0·05
iso-C4%	—	—	3[a]	1[d]	3[b]	2[c]	0·2	0·001	0·001	0·001
C4%	—	—	7[b]	8[a]	5[c]	7[b]	0·2	0·001	0·01	NS
iso-C5%	—	—	2[a]	1[b]	1[b]	1[b]	0·1	0·001	0·001	0·01
C5%	—	—	2[a]	2[a]	1[b]	2[a]	0·1	0·001	NS	0·001

[a, b, c, d] Mean values within the same horizontal line with different superscript letters were significantly different (Newman–Keuls test): $P < 0·05$.

nd, not detected.

* For details of diets and procedures, see pp. 904–905

DISCUSSION

Results for mucin-containing cells are expressed in terms of positive response to PAS, AB 2·5 and HID staining; i.e. related to the presence of neutral mucin, acid mucin (both sialylated and sulphated) and sulphomucins (strictly sulphated mucins) respectively.

It has been shown in pathological studies that there are qualitative and/or quantitative alterations in epithelial mucins of human adenocarcinomas (Filipe, 1979) or colitis (Smith & Podolsky, 1986). However, no significant correlation was found between faecal mucus-degrading glycosidases and ulcerative colitis and Crohn's disease (Rhodes *et al.* 1985*a*, *b*). The role of colonic mucus and mucosal glycoproteins was reviewed recently (Rhodes, 1989). Some bacterial strains efficiently metabolize mucus as a substrate *in vitro* (Gibson *et al.* 1988). Even though our study only concerns nutritional effects, it is of interest to determine whether the diet and/or the bacterial flora may alter mucin composition totally or partially, leading to variations in neutral, acid, and strictly sulphated mucin ratios.

In rats the number of mucus-containing cells varied according to the absence of bacteria and the bacterial status, the anatomical site and the diet composition.

In the caecum of GF rats the number of mucus cells was lower than that in CV rats, as shown previously by Ishikawa *et al.* (1986, 1989). However, little is known about the mucin composition of rats except that the amino acids of mucus in caecal contents of GF rats are different from those of their CV counterparts (Combe *et al.* 1976). In our experiment the number of AB 2·5-positive cells was lower than those of PAS- and HID-positive cells in GF compared with CV rats.

The greater extent of staining of the AB 2·5-positive subepithelial lumen mucin layer in the caecum and the released mucus still attached to the epithelial goblet cells in the GF distal colon confirm the fact the mucus was not washed away; this finding may be related to the well-known impaired transit time of the intestinal contents in the GF condition.

In the caecum of the GF rat, the small number for AB 2·5-positive cells in the crypts

(Table 1) and the observation of an increased extent of staining from the subepithelial lumen mucus layer to the intraluminal caecal contents (Table 2) suggested that acid mucin is secreted from goblet cells located in the crypts and may explain this very small number for acid-mucin-containing cells. These observations were similar with or without TOS in the diet.

In the caecum of the CV rat, despite a greater number of AB 2·5-positive cells in the crypts, as compared with the GF rat (Table 1), the extent of staining of the subepithelial lumen mucus layer was weaker than that in GF rats (Table 2), both with or without TOS in the diet. However, intraluminal caecal contents were highly stained. The weaker staining of the subepithelial luminal mucus layer, especially with TOS in the diet, for acid mucin and sulphomucin might be due to the activity of bacteria located in this mucus layer, and the role of bacterial mucin sulphatases could be involved in this effect.

In GF rats the number of mucus-containing cells increased from the caecum to the colon. A similar finding was observed only for acid mucin in CV rats. In the distal colon of both types of rats the proportion of the three types of mucin appeared similar. In the proximal colon acid mucin remained slightly lower in GF rats than in CV rats. On the contrary, recent work with mice reported more acid-mucin-containing cells in the ascending colon of GF compared with specified pathogen-free counterparts (Hill & Cowley, 1990), suggesting a bacterial specificity for mucin distribution.

TOS ingestion had no effect in the caecum of GF rats but it modified the mucin cell distribution in the colon. The lower number of mucus cells observed here for the three types of mucins in the proximal colon and the greater number in the distal colon may be related to the fact that TOS may act in the large intestine of GF rats by modifying the osmolality of contents. It had been demonstrated by Sakata & Engelhardt (1981*a*, *b*) using solutions of SCFA of variable osmolalities that the lowest osmolality caused a considerable release of mucins from goblet cells both in the proximal and distal colon, and that the lower the osmolality the larger the mucin release.

In CV rats TOS had no effect in the colon, whereas it decreased the number of acid-mucin-containing cells in the caecum. This difference may be due to the presence of a higher SCFA concentration in caecal contents compared with colon contents (Rémésy & Demigné, 1976).

It was of interest to observe that human bacterial flora could hydrolyse rat mucin, since sugar constituents are similar, despite structural differences reported between rat and human mucins (Allen, 1981). The mucolytic activities obtained were of the same magnitude in HE and CV rats fed on the control diet, showing that the human flora can hydrolyse rat mucin.

However, fermentative bacterial metabolism was not similar in HE and in CV rats; CV rats produced more gas and had higher caecal SCFA concentrations than HE rats. Similar to the observations of Debure *et al.* (1989), the proportion of acetic acid was higher and that of propionic, butyric and valeric acids lower in HE rats than in CV rats. Nevertheless these authors observed similar total SCFA concentrations in HE rats and in CV rats. A previous comparison between two groups of rats inoculated with two different human floras from methane and non-methane producers respectively has shown large differences in caecal SCFA concentrations and in L-lactic acid: D-lactic acid values (Andrieux *et al.* 1991). The differences observed in the present experiment might be due to the specificity of the bacterial microflora, since it has been shown that the human flora inoculated into GF rats keeps its major properties; specified bacterial populations, enzymic activities and fermentative profile (Mallett *et al.* 1987; Debure *et al.* 1989; Andrieux *et al.* 1991).

Despite the non-significant increase in caecal SCFA concentrations, the reduction of caecal pH in CV and HE rats and the modifications of the metabolite profile, especially the

increase in gas production, confirm TOS fermentation in the large intestine. However, no evident relationship between the mucin-containing cell distribution and the bacterial metabolites studied (gas, SCFA, D- or L-lactate) was shown. In order to elucidate the relationship between mucin distribution and specific mucolytic bacterial activities it would be of interest to study gnotoxenic rats associated with bacteria chosen for their specific mucolytic activities and fed on fermentable carbohydrates.

In conclusion, TOS affected mucus cell distribution in the colon of GF rats. The presence of the flora, conventional or human, abolished this effect.

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