Intestinal mucin distribution in the germ-free rat and in the heteroxenic rat harbouring a human bacterial flora: effect of inulin in the diet

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A colorimetric method was used on water-soluble mucin extracted from mucosal scrapings and contents of the caecum and the colon of five germ-free (GF) rats and five heteroxenic (HE) rats harbouring a human flora (GF rats associated with a human flora). These rats were fed on a diet containing either 100 g sucrose/kg or 100 g inulin/kg. Histological stains, periodic acid–Schiff, alcian blue pH 2·5 and alcian blue pH 0·5 were used to discriminate between neutral, acidic and acidic sulphated mucins respectively. Spectrocolorimetric assays led to a calculated absorbance value for 1 mg of the initial mucin extract. Each mucin type was compared between treatments. The caecal contents of GF rats contained more acidic mucin than sulphomucin, which was present in the same proportion as neutral mucin. Their colonic contents contained more acidic mucins than sulphomucin, which in turn was more abundant than neutral mucin. Their caecal mucosa mucin distribution differed from that of the contents: very little acidic mucin was present and neutral and sulphomucin proportions were of the same order of magnitude. Inulin increased the amount of neutral mucin in the caecal contents and of sulphated mucins in the colonic contents and increased the amounts of neutral and acidic mucins in the caecal mucosa. Mucin distribution in the HE rats was very different from that in the GF rats: the caecal contents contained a high proportion of acidic mucins and very little sulphomucin. The same distribution of mucins was observed in the colonic contents. The caecal mucosa contained less acidic mucin and more sulphomucin than the caecal contents. Inulin decreased acidic mucins and increased sulphated mucins in the caecal contents and increased neutral and sulphated mucins in the colonic contents. Inulin increased sulphomucin in the caecal mucosa and decreased acidic mucin in the caecal and colonic mucosas. The very low amount of mucin that was recovered in the colonic mucosa suggests that, in the presence of the bacterial flora and associated with inulin in the diet, mucin was extensively released from the mucosa to the colonic lumen. This might be related to the bacterial metabolites produced.

Mucins: Intestine: Bacteria: Inulin

Mucin glycoproteins can be classified into three groups on the basis of their histochemical staining: neutral mucins, sialic acid-containing mucins and ester sulphate-containing mucins (Filipe, 1979). The presence of sulphate and sialic acids on the carbohydrate chains confers to the intestinal acidic mucins physicochemical properties different from those of neutral mucins, resulting in higher viscosity and acidity (Allen *et al.* 1982). These acidic mucins increase the mucus potential to resist attack by bacterial enzymes (Rhodes, 1989). In man, neutral, acidic and sulphated mucins vary widely in various diseases (Filipe, 1979; Smith & Podolsky, 1986). In animals, age and diet affect the composition of the intestinal mucins (Satchithanandam *et al.* 1990; Turck *et al.* 1993). Dietary fibres have been shown to alter intestinal mucus composition qualitatively and/or quantitatively (Cassidy *et al.* 1981; Huang *et al.* 1990) either by a mechanical effect on the intestinal mucosa (Komai &

Kimura, 1980; Ecknauer *et al.* 1981; Southon *et al.* 1985; Dirks & Freeman, 1987), or by the bacterial fermentation metabolites acting on the mucosal metabolism. End-products of fermentation are mainly short-chain fatty acids (SCFA), which may induce local osmolality variations resulting in mucus secretion (Sakata & Engelhardt, 1981). Furthermore, dietary fibres induce the activity of glycolytic enzymes, which could alter mucin degradation (Salyers *et al.* 1977).

In a previous paper, by using a technique based on the histochemical characteristics of the mucin types we showed, in the rat, that the bacterial flora modified the goblet-cell mucin distribution in the caecal and colonic mucosas (Meslin *et al.* 1993). Supplementing the diet with poorly digestible and fermentable carbohydrate was without effect on caecal mucin composition in the germ-free (GF) rat, but modified goblet-cell number and mucin types in conventional and heteroxenic (HE) rats. Studies have shown that human flora inoculated into GF rats keeps its major properties, specific bacterial populations, enzymic activities and fermentative profile (Andremont *et al.* 1985; Cole *et al.* 1985; Mallett *et al.* 1987; Debure *et al.* 1989; Andrieux *et al.* 1991). The HE rat mimetic model obtained is more valuable than the conventional rat for studying colonic fermentation in man. Comparing GF rats with HE rats provides information on the action of the intestinal microbial flora.

The aim of the present study was to investigate the effect of a bacterial flora on the proportions of mucin types both in the luminal contents and in the mucosa of the caecum and colon in the rat. The diet was supplemented with inulin (100 g/kg), in order to increase bacterial metabolite production. Inulin occurs in cereals, onions, artichokes and other compositae (Bacon & Edelman, 1951). It is composed of 15–30 α 1-2 fructose units, and is thus indigestible by endogenous enzymes and completely fermented by the microbial flora (Nilsson & Björck, 1988; Nilsson *et al.* 1988; Rumessen *et al.* 1990). In HE rats the fermentation of inulin lowers the pH of caecal contents and increases SCFA concentrations (Andrieux *et al.* 1991), particularly butyrate, which has a trophic effect on the intestinal mucosa (Sakata, 1987).

MATERIALS AND METHODS

Animals

Male GF and HE inbred F344 rats (2 months old) were used. HE rats were obtained by inoculating GF rats with human faecal flora. Human faeces provided by a methano-producer were diluted 1:20 (w/v) with saline (9 g NaCl/l) in an anaerobic chamber; 1 ml of this dilution was inoculated orally twice during 24 h. Animals were cared for in accordance with the guidelines set out by our institute and adapted to conform to those established by the Canadian Council for Animal Care (Ottawa, Ontario, 1984). 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); one isolator was used for each bacterial status.

Diets

Two diets were used: a control diet containing (g/kg) cooked potatoes 460, fish meal 230, lard 100, cellulose 50, maize oil 40, mineral and vitamin mixture (composition previously described by Andrieux & Sacquet, 1986) 20, and sucrose 100, and an experimental diet in which 100 g sucrose/kg was replaced by 100 g inulin/kg extracted from chicory and provided by ARD, Paris, France. Sterilized feed (γ irradiation, 45 kGy in vacuum-sealed plastic bags) and water were given *ad libitum* to the rats.

Experimental design

Groups of ten GF rats and ten HE rats were used; five rats were fed for 1 month on the control diet, five on the experimental diet. At the end of this adaptation period the rats were killed by an overdose of sodium pentobarbitone (SANOFI, Aulnay/Bois, France: 60 mg/kg intraperitoneally). Caecal pH was measured. The caecum and colon were then removed with their contents. A portion of the caecal contents (1 g) was frozen with liquid N_2 and maintained at $-20°$ until required for metabolite determination. For each group of rats the remaining caecal contents and colonic contents were separated from the mucosa by injecting into the hindgut lumen a NaCl (9 g/l)–EDTA (2 mM) buffer containing bacterial protease inhibitors (Na azide 0·02 g/l, phenylmethylsulphonyl fluoride 0·1 mM) and pooled. The mucosas from the caecum and the colon were scraped with a histological slide and collected separately. The water-soluble mucin fractions were extracted from the pooled caecal and colonic contents and from caecal and colonic mucosal scrapings.

Extraction of water-soluble mucins from the samples

The methodology already described (Fontaine & Meslin, 1994) was slightly modified from Miller & Hoskins (1981). Each sample was homogenized, then centrifuged. The supernatant fraction was precipitated with ethanol; the pellet was dialysed against distilled water and lyophilized. The weight and protein composition of the lyophilized powder were determined by a modified Lowry method (Bensadoun & Weinstein, 1976). The total weight of mucin was corrected by taking into account the 1 g of caecal contents removed for metabolite determination.

Neutral, acidic (sialomucins and sulphomucins) and strictly sulphated mucins (sulphomucins) were characterized by transposing the tests used in histochemistry to the mucins of the intestinal globlet cells, as previously described (Fontaine & Meslin, 1994). The initial samples consisted of 1 mg of the lyophilized powder dissolved in 1 ml of distilled water. This was diluted to 0·025–0·150 mg/ml for neutral mucin determination and to 0·1–0·5 mg/ml for acidic and sulphated mucins; histochemical reagents (periodic acid–Schiff (PAS) pH 7, or alcian blue (AB) pH 2·5 or AB pH 0·5 respectively) were added, and the pH adjusted as required. Acidic mucins were precipitated in the presence of modified Carnoy fixative (ethanol (950 ml/l)–formaldehyde (350 ml/l)–acetic acid (6:3:1, by vol.)). Centrifugation was carried out at 9500 ***g*** for 10 min. The pellet was dissolved at the convenient pH in the presence of Triton X100 and sonicated for 1 min (Bransonic 52 tank, Biobloc Scientific, Paris, France). Absorbance was read with a Shimatsu spectrophotometer (Roucaire, Vélizy, France) at 600 nm for the AB and at 570 nm for the PAS reaction.

Interference by dietary compounds

Interference of dietary compounds present in the intestinal luminal contents was tested by treating a portion of the diet in the same manner as the intestinal contents or mucosal scrapings.

Electrophoretic controls

As in the previous paper (Fontaine & Meslin, 1994), electrophoretic controls were used (results not shown) to verify that protein contaminants, if present, did not interfere with mucin determination.

Bacterial metabolite analysis

SCFA were analysed, using GLC after water extraction of acidified samples (Andrieux *et al.* 1991). D and L-lactic acids were determined enzymically (UV Boehringer method, Meylan, France).

Table 1. *Body weight, caecal weight and pH, and short-chain fatty acid (SCFA) composition in germ-free and heteroxenic rats fed on control or inulin-containing diets**

(Mean values with their standard errors for five rats per group)

Diet...	Germ-free rats				Heteroxenic rats			
	Control		Inulin		Control		Inulin	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body wt (g)	329[a]	27	300[a]	12	311[a]	12	308[a]	9
Caecal wt (g)	16·2[a]	2·5	16·1[a]	1·5	3·7[c]	0·4	6·5[b]	0·7
Caecal pH	7·6[a]	0·1	7·3[a]	0·1	6·6[b]	0·1	6·3[c]	0·1
Total SCFA (μmol)	40·5[c]	3·5	37·0[c]	2·0	104·7[b]	3·6	221·0[a]	1·7
SCFA (μmol/g caecal content)	2·5[c]	1·0	2·3[c]	0·5	28·3[b]	3·2	34·0[a]	1·0
Acetate (%)	100		100		64		64	
Propionate (%)	—		—		21		22	
Butyrate (%)	—		—		7		12	
Valerate (%)	—		—		2		0	
Isoacids (%)	—		—		6		0	
Total lactate (μmol)	—		—		7·4[b]	0·5	17·6[a]	1·1
Lactate (μmol/g caecal content)	—		—		2·0[b]	0·1	2·7[a]	0·3
L-Lactate (%)	—		—		71		61	
D-Lactate (%)	—		—		29		39	

[a, b, c] Mean values within a row with unlike superscript letters were significantly different, $P < 0.05$.

* For details of diets and procedures, see pp. 882–883.

Table 2. *Weight and protein content of lyophilized mucin powder from the caecal and colonic contents and mucosa of germ-free and heteroxenic rats fed on control or inulin-containing diets**

	Weight (mg)	Protein content (mg)	Mucin (mg/mg protein)
Germ-free rats (*n* 5)			
Contents			
Caecum, control diet	283	2·6	108·8
Caecum, inulin diet	266	3·4	78·2
Colon, control diet	34	0·7	48·6
Colon, inulin diet	34	0·5	68·0
Mucosa†			
Caecum, control diet	6·0	1·2	5·0
Caecum, inulin diet	6·6	1·8	3·7
Heteroxenic rats (*n* 5)			
Contents			
Caecum, control diet	46·4	8·8	5·3
Caecum, inulin diet	58·6	10·2	5·7
Colon, control diet	22·5	4·0	5·6
Colon, inulin diet	25·0	5·0	5·0
Mucosa			
Caecum, control diet	39·9	27·5	1·5
Caecum, inulin diet	40·0	28·8	1·4
Colon, control diet	106·3	73·5	1·4
Colon, inulin diet	2·5	1·5	1·7

* For details of diets and procedures, see pp. 882–883.

† Colonic mucosas were not determined in the germ-free group due to technical difficulties.

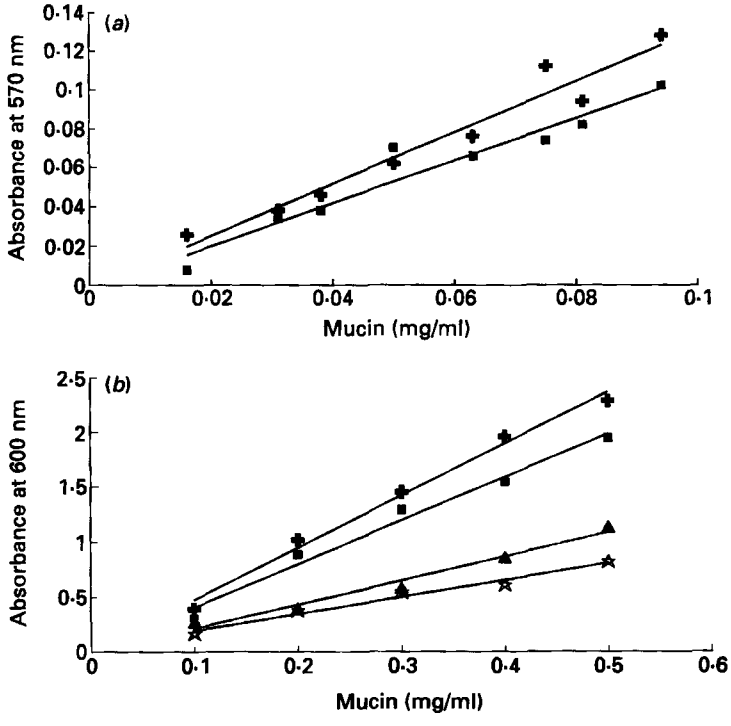

Fig. 1. Spectrocolorimetric assays of (a) neutral mucin and (b) acidic and sulphated mucins in the colonic contents of germ-free rats fed on a control diet or an inulin-containing diet. Panel (a): (■), neutral mucin, control; (✚), neutral mucin, inulin. Panel (b): (■), acidic mucin, control; (✚), acidic mucin, inulin; (☆), sulphated mucin, control; (▲), sulphated mucin, inulin.

Statistical analysis

For each group the results from individual rats were expressed as means with their standard errors. Experimental values were compared by ANOVA and Newman–Keuls multiple-range tests; the slopes of the assays for each mucin type were compared by linear regression coefficient analysis (STATITCF software; ITCF, Paris, France).

RESULTS

Body weight, caecal weight and caecal metabolite concentration (Table 1)

Body weight was similar in all groups (mean 312 (SE 10) g). Germ-free rats were characterized by a large caecum (16 g), an approximately neutral caecal pH and a very low SCFA concentration (2·5 μmol acetate/g). Inulin in the diet did not modify these values.

In HE rats fed on the control diet the mean caecal weight was only 3·7 g, the caecal pH was slightly acidic (6·6), the concentration of SCFA was about 28 μmol/g and that of lactic acid 2 μmol/g caecal content. Inulin in the diet doubled the caecal weight in HE rats, significantly reduced the pH (to 6·3) and significantly increased the amount and concentration of total SCFA and lactic acid. The SCFA and lactic acid profiles also changed: the proportion of butyrate was enhanced whereas valerate and caproate disappeared and the proportion of L-lactic acid was lowered.

Table 3. *Mucin distribution in contents and mucosa from the caecum and colon of germ-free and heteroxenic rats fed on control or inulin-containing diets**

(Calculated slopes of the spectrocolorimetric assays from 1 mg lyophilized mucin powder)

Mucin type...	Neutral (PAS+)	Acidic (AB 2·5+)	Sulphated (AB 0·5+)
Germ-free rats			
Contents			
Caecum, control diet	1·25	4·50	1·33
Caecum, inulin diet	1·59	4·08	1·42
Colon, control diet	1·09	4·02	1·67
Colon, inulin diet	1·32	4·73	2·19
Mucosa†			
Caecum, control diet	1·89	1·77	1·74
Caecum, inulin diet	2·87	2·34	1·88
Heteroxenic rats			
Contents			
Caecum, control diet	3·10	15·3	0·34
Caecum, inulin diet	3·44	12·0	0·42
Colon, control diet	2·40	15·4	0·66
Colon, inulin diet	3·70	13·8	0·85
Mucosa			
Caecum, control diet	2·82	5·48	0·80
Caecum, inulin diet	2·50	2·60	1·03
Colon, control diet	1·80	4·03	0·80
Colon, inulin diet	2·70	1·82	1·34

PAS, periodic acid-Schiff reagent; AB, alcian blue reagent.

* For details of diets and procedures, see pp. 882–885.

† Colonic mucosas were not determined in the germ-free group due to technical difficulties.

Amounts of mucin powder and corresponding proteins in caecal and colonic luminal contents and in mucosas (Table 2)

The amount of crude mucin obtained from the caecal contents of GF rats (283 mg) was fivefold higher than in HE rats (46·4 mg). On the other hand, a lower amount of mucin was found in the caecal mucosa of GF rats: 6 mg, instead of 40 mg in the caecal mucosa of HE rats. It should be noted that we could not obtain enough powder for analysis from the colonic mucosa in the groups of five GF rats. Inulin in the diet tended to decrease the amount of mucin in caecal contents of GF rats but enhanced that of HE rats; it did not modify the amount of mucin in the caecal mucosa. The colon of GF rats, like the caecum, contained more mucin than the colon of HE rats. Inulin had no effect on the amount of mucin in the colonic luminal contents but it reduced strongly the amount of mucin in the mucosa of HE rats.

The mean ratios (w/w) of protein expressed per total mucin weight were 23·5 and 67·5% in mucosal scrapings from GF and HE rats respectively. They were tenfold lower in caecal and colonic contents of GF rats and fivefold lower in these samples from HE rats. Protein contents of the mucin samples were not affected by the diet.

Mucin-type determination

Dietary inulin still present in the caecal and the colonic contents was washed away during dialysis. The artefact in the mucin assay due to other residual dietary compounds was 0·01 absorbance units/mg for the neutral mucin, 0·02 absorbance units/mg for the acid mucin

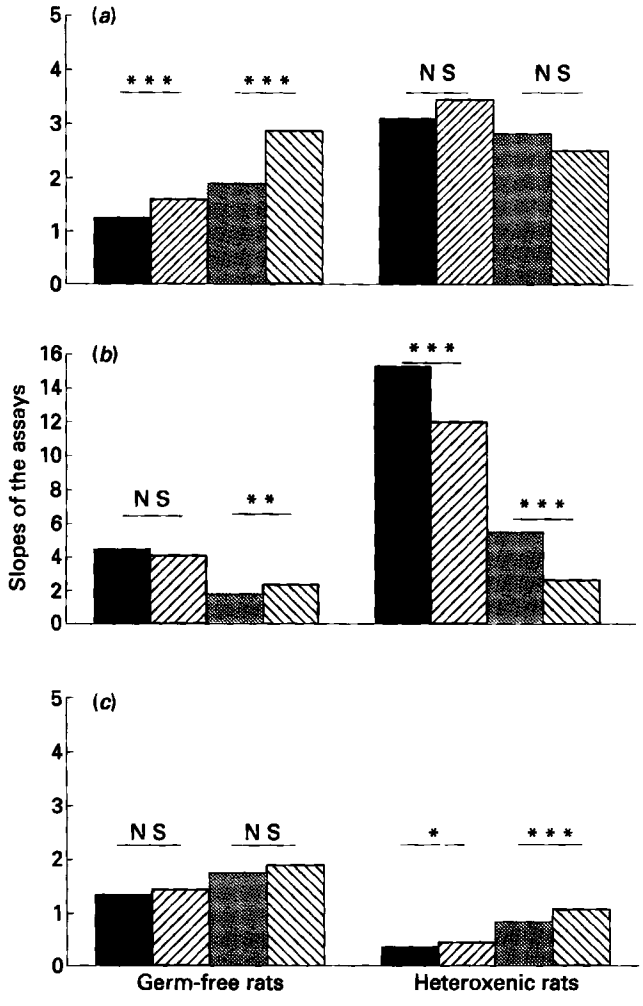

Fig. 2. Slopes of the assays of (a) caecal neutral mucins, (b) caecal acidic mucins and (c) caecal sulphated mucins in the contents (■, ▨) and mucosa (▩, ▧) of germ-free and heteroxenic rats fed on a control diet (■, ▩) or an inulin-containing diet (▨, ▧). Mean values were significantly different for the two diets: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and 0·05 absorbance units/mg for the sulphated mucin. The very small size of these values did not affect comparison between the two diets, particularly for acidic and sulphated mucins (Fig. 1(b)).

Fig. 1 shows assays of neutral (a), and acidic and sulphated (b) mucins in the colonic contents of the GF rats fed on control or inulin-containing diets. All other samples were treated in the same manner (results not shown). There was a linear relationship between absorbance and the semi-purified mucin concentration (mg/ml) for each mucin type characterized. The slopes of the curves were calculated from these results (Table 3). Bar graphs (Figs. 2 and 3) compare each mucin between experimental treatments (e.g. inulin diet *v.* control diet in the contents or in the mucosa) in GF or HE rats, and refer to the absorbance for 1 mg mucin.

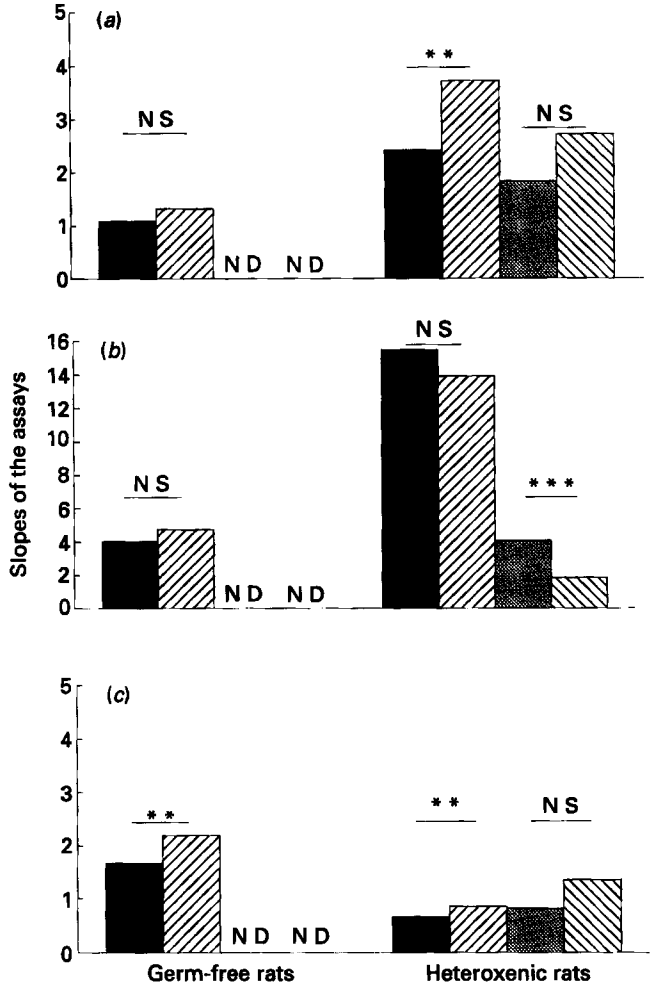

Fig. 3. Slopes of the assays of (a) colonic neutral mucins, (b) colonic acidic mucins and (c) colonic sulphated mucins in the contents (■, ▨) and mucosa (▩, ▧) of germ-free and heteroxenic rats fed on a control diet (■, ▩) or an inulin-containing diet (▨, ▧). Mean values were significantly different for the two diets: ** $P < 0.01$, *** $P < 0.001$. ND, not determined.

Caecal neutral mucins in GF rats were significantly increased both in the contents and in the mucosa under an inulin-containing diet. Caecal contents of HE rats contained three times as much caecal neutral mucin as those of the GF group, whereas inulin affected neither the contents nor the mucosa (Fig. 2(a)).

The variations of caecal acidic mucins (Fig. 2(b)) were very different from those of neutral mucins. In GF rats, inulin had no effect on the contents, and significantly increased acidic mucins in the mucosa, although this increase was low. Values in HE rat contents were about four times as high as those in GF rats, and dietary inulin significantly decreased acidic mucins in both the contents and the mucosa (Fig. 2(b)).

The caecal sulphated mucins (Fig. 2(c)) in GF rats were not modified by dietary inulin either in the contents or in the mucosa. HE rats responded differently: dietary inulin increased sulphated mucins both in the contents and in the mucosa; however, this increase

was larger in the mucosa than in the contents. The values in the HE rat contents and mucosa were twice as low as in the GF rats.

Inulin did not affect the amount of colonic neutral mucin in the contents of the GF rats (Fig. 3(a)). On the other hand, it significantly increased neutral mucins in the colonic contents of HE rats but did not affect their mucosa. Colonic content values were twice as high in HE rats as in GF rats.

The colonic acidic mucins (Fig. 3(b)) in the GF rat contents did not differ from the caecal acidic mucins and dietary inulin was without effect. In the HE rats the control diet yielded colonic content values similar to the caecal content values. Inulin had no effect on the acidic mucins in the contents, but reduced significantly the acidic mucins in the colonic mucosa, similarly to what was observed in the caecal mucosa.

The effect of inulin on the colonic sulphated mucins (Fig. 3(c)) in the contents of the GF rats was different from that observed in the caecum: sulphated mucins increased significantly. In the HE rats sulphated mucins increased significantly in the colonic contents under an inulin diet whereas inulin did not affect sulphated mucins in the mucosa.

DISCUSSION

Our results show that the amount of mucin and its distribution according to histochemical staining differed strongly between GF and HE rats, both within luminal contents and in the mucosa. The greater amount of mucin collected from the caecal contents of the GF rats as compared with that of HE rats (Table 2) confirms the previous findings of Hoskins & Zamcheck (1968). This is due to the lack of degradation, i.e. of mucolytic activity, in the absence of microflora, as shown previously (Meslin *et al.* 1993). The accumulation of mucin is believed to hinder water absorption and contribute to the caecal enlargement (Asano, 1967; Donowitz & Binder, 1979). However, although the caecal volume of GF rats was five time as large as in HE rats (Table 1), the amount of mucin in the present experiment was also five times as large. This suggests that caecal mucin concentrations are quite similar in GF and HE rats. Moreover Enss *et al.* (1992) found similar amounts of mucin in colonic contents of GF and specific-pathogen-free rats.

In a previous study using a pilocarpin treatment, Fontaine & Meslin (1994) collected the intestinal mucin of GF rats from both mucosa and lumen of the small intestine and of the hindgut. Mucin was distributed into the following types (absorbance units/mg): 3·26 neutral mucin, 4·43 sialomucin and 1·85 sulphomucin. Furthermore this mucin was in fact a mixture of mucin discharged from the mucosa to the lumen as a result of pilocarpin treatment and of mucin normally present in the luminal contents collected from the small intestine and the hindgut. Therefore, the mucin type proportions in this previous study, like those of the present study, differed according to anatomical site and to whether mucosal or luminal contents were considered. Colonic mucin in several species differs from mucin in the proximal intestine by its greater proportion of acidic mucin (Allen *et al.* 1982). This suggests that, because bacterial degradation was absent in GF rats, the local composition of luminal mucin represents both the local secretion from the mucosa and the accumulation of the fraction secreted from the upper parts of the gastrointestinal tract.

The caecal contents of GF rats in the present study also contained more acidic mucins than sulphated mucins. This was due to sialomucins: the AB pH 2·5 reaction we used detected both sialylated and sulphated mucins and the AB pH 0·5 reaction only the strictly sulphated mucins. Sialomucins might be obtained as the difference between these two values. The caecal and colonic mucosas of GF rats were characterized by a very low amount of mucin and low number of mucin-containing cells (Meslin *et al.* 1993).

The mean percentage of protein in the caecal mucosal scraping of GF rats was similar

to the value found by Slayter *et al.* (1991). Surprisingly, we found a much lower percentage of protein in caecal and colonic contents of GF rats. As endogenous proteases efficiently hydrolyse mucin proteins (Allen *et al.* 1982; Pasquier & Vatier, 1990), this may be due to the greater endogenous proteolytic activity in the large intestine of GF rats than in conventional rats (Reddy *et al.* 1968).

Our mucin preparation was not contaminated by carbohydrates from the diet present in the intestinal contents, since very little staining was found when diet was treated as mucin. Mucolytic activities were found in the caecal content of HE rats showing that bacteria from humans can degrade carbohydrate chains of rat mucin (Meslin *et al.* 1993). Consequently, the luminal mucin composition in HE rats resulted from local and proximal intestinal secretions, as in GF rats, in addition to bacterial degradation. It has been shown that mucin plays an important role as a bacterial substrate in the colon (Flourié *et al.* 1991) and that many bacteria from the intestinal microflora are capable of degrading mucus glycoproteins (Salyers *et al.* 1977). Some bacteria such as *Bifidobacterium bifidum* use mucus as their sole source of energy (Miller & Hoskins, 1981) whereas the mucin pattern is not affected by others such as *Peptostreptococcus* when they are inoculated into the GF rat (Gustafsson *et al.* 1981).

Staining with AB pH 2·5, which stains total acidic mucins, was more intense in HE rats than in GF rats. More intense staining was also observed for neutral mucins, but not for sulphated mucins. Szentkuti *et al.* (1990), using histochemical methods, observed a stronger stainability of all mucin types in conventional rats than in GF rats, whereas Enss *et al.* (1992), using ion-exchange chromatography, confirmed that relative amounts of acidic mucins are higher in specific-pathogen-free rats.

Results obtained with the inulin diet confirm that intestinal fermentation, which did occur as demonstrated by the metabolites produced (Table 1), can alter the composition of mucosal mucins. Adding inulin to the diet of GF rats increased caecal neutral mucins both in the contents and mucosa, increased caecal acidic mucins in the mucosa, and increased colonic sulphated mucins in the contents. Adding inulin to the diet of HE rats decreased caecal acidic mucins both in the contents and in the mucosa. This decrease affected sialomucins since sulphated mucins were slightly enhanced. Inulin increased neutral mucins in the contents of the HE rat colon, decreased acidic mucins in its mucosa and increased sulphated mucins in its contents. These variations might be related to the bacterial metabolites produced in the contents and absorbed by the mucosa. Inulin actually decreased the amount of mucin in the colonic mucosa (Table 2). This suggests that inulin fermentation induced a release of mucin into the gut lumen. Inulin increased total SCFA and total lactate, thereby significantly reducing the pH (Table 1). Acidification of intestinal contents has been shown to stimulate mucus secretion, while SCFA behave in a more complex way. Sakata & Engelhardt (1981), using solutions of SCFA of various osmolalities, demonstrated that low osmolality causes a considerable release of mucins from goblet cells both in the proximal and distal colon, and that the lower the osmolality the larger the amount of mucin released. However, the main effect of the inulin diet was an increase of sulphomucin together with a reciprocal decrease of sialomucin in the caecal and colonic mucosas. These variations are important to consider, as it has been reported that sulphomucin can increase potential resistance to attacks by bacterial enzymes (Rhodes, 1989). In GF rats, Enss *et al.* (1994) recently observed that a mechanical challenge causes alterations of rat colonic mucosa and released mucins. In conventional rats fed with several insoluble or soluble dietary fibres, Cassidy *et al.* (1990) and Satchithanandam *et al.* (1990) also observed an increase in the proportion of sulphomucin and the amount of total mucin in the small intestine and in the colon. These authors postulated that the change in mucin composition contributed to the protective effect of dietary fibres in the incidence of colon

cancer. In conclusion, inulin may have a beneficial effect on health by stimulating sulphomucin synthesis; however, a low amount of mucin in the colon may be considered less beneficial for the mucosal physiology.

Ph Butler (INRA translation and terminology service) improved the English manuscript.

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