Effects of some poorly digestible carbohydrates on bile acid bacterial transformations in the rat

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The effects of ingestion of poorly digestible carbohydrates on bacterial transformations of cholic acid and β-muricholic acid were studied in rats fed on increasing levels of lactose, lactulose, amylomaize or potato starches. Each level was given for 3 weeks and, at the end of each dietary treatment, bile acid faecal composition was analysed and a group of six rats was killed every 4 h during 24 h to determine the amounts of fermented carbohydrate and fermentation characteristics (caecal pH, volatile fatty acids (VFA) and lactic acid concentrations). Fermentation of carbohydrates decreased caecal pH and enhanced caecal VFA and lactic acid concentrations. Irrespective of the poorly digestible carbohydrate, the variation of bacterial transformation always occurred in the same way: the bacterial transformation of β-muricholic acid into hyodeoxycholic acid was the first to disappear, while ω-muricholic acid formation increased; second, cholic acid transformation decreased and finally all bile acid transformations were strongly affected. There was a significant correlation between bile acid transfer and the minimal caecal pH in vivo. This effect of pH was similar in vitro. To determine whether the levels of bacteria which transformed bile acids were modified, rats fed on the highest amounts of poorly digestible carbohydrates were introduced into isolators and carbohydrate feeding was stopped. Caecal pH recovered its initial value but bile acid transformations remained changed, suggesting that the intestinal microflora were modified by ingestion of fermentable carbohydrates.

Bacterial transformation: Bile acids: Carbohydrates: Rat

The microbial flora of the large intestine ferment poorly digestible carbohydrates. It is assumed that these fermentations modify the intestinal microflora. However, quantitative microbiological analyses have often shown that the diet has little effect on the bacterial population (Moore & Holdeman, 1975; Drasar *et al.* 1976; Bornside, 1978; Ducluzeau *et al.* 1984), whereas Finegold *et al.* (1974, 1977) have demonstrated that a high-meat, low-fibre diet increases the anaerobic bacterial population. The number of bacterial species and the difficulty of growing some species may explain this controversy. Another approach involves studying the effect of carbohydrates on microbial metabolism. In man and animals a protein-rich, low-indigestible-carbohydrate diet increases some bacterial enzyme activities such as bile acid transformations. In contrast, a lacto-vegetarian or vegetarian diet reduces these bacterial enzyme activities (Reddy & Wynder, 1973; Nigro *et al.* 1976; Reddy *et al.* 1980). However, experimental results show a large variability in the effects due to dietary carbohydrate. Thus, wheat bran and cellulose reduce the proportion of deoxycholic acid in human bile (Pomare & Heaton, 1973; Hillman *et al.* 1986; Nomani *et al.* 1986), whereas pectin leads to the reverse effect and lignin has no effect (Hillman *et al.* 1986). In rats, bran does not modify bacterial bile acid transformations and amylomaize starch reduces them considerably (Sacquet *et al.* 1983).

The mechanism of action of these dietary fibres has not been well established, in particular the role of the various products of fermentation and that of the decrease in

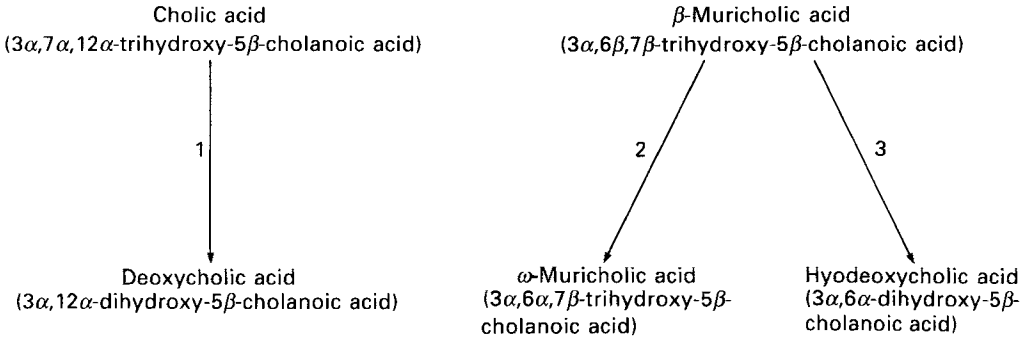

Fig. 1. Important transformations of cholic and β-muricholic acid by micro-organisms in conventional rats. 1, 7α-dehydroxylase (Gustafsson, 1966); 2, 6β → 6α epimerase (Sacquet *et al.* 1979*b*); 3, mechanism discussed by Van Heijenoort *et al.* (1974), Sacquet *et al.* (1979*a*) and Eyssen *et al.* (1985).

intestinal pH. It has not been demonstrated whether fermentation modifies the bacterial transformations of bile acids (1) because intestinal pH differs from the optimal pH for enzyme activity, (2) the fermentation products exert a specific anti-bacterial effect or (3) the number of bacteria and, therefore, concentrations of enzymes are reduced in the intestine.

In male germ-free rats, the major bile acids synthesized are cholic acid and β-muricholic acid (Kellogg & Wostman, 1969). In conventional rats the transformations of these bile acids by micro-organisms lead to the production of a variety of metabolites (Hayakawa, 1973). The most important transformations are presented in Fig. 1.

The present work was designed to determine in rats (1) the fermentation characteristics of four poorly digestible carbohydrates: lactose, lactulose, amylomaize starch and potato starch, (2) the bacterial transformations of the two major bile acids synthesized by the rat (cholic and β-muricholic acids), (3) the relation between fermentation characteristics and enzyme activities.

To determine whether the concentrations of bacteria which transform bile acids are modified, rats were introduced into isolators, fermentable carbohydrate ingestion stopped and the possible restoration of bile acid bacterial transformations studied.

MATERIALS AND METHODS

Animals and diets

The control semi-synthetic diet contained (g/kg): maize starch 580, casein 205, maize oil 90, cellulose 50, minerals and vitamins 75 (Andrieux & Sacquet, 1986). In experimental diets, increasing amounts of lactose, lactulose (Duphalac; Duphar), amylomaize starch (Eurylon; Roquette frères, Lestrem) or potato starch (Roquette frères) were substituted for maize starch.

The experimental diets contained 40, 80, 160, 320 or 480 g lactose/kg; 40, 80 or 160 g lactulose/kg; 160, 320 or 480 g amylomaize starch/kg; 160, 320 or 480 g potato starch/kg.

Expt 1. Seventy-eight 7-week-old male rats of Fischer strain 344 were fed on the control diet for 2 weeks. At the end of the second week, faeces were collected for 3 d for bile acid analysis. Six rats were killed constituting the first control group (C1). In this group, one rat was killed every 4 h for 24 h. The amount of fermented carbohydrate (i.e. maize starch), caecal weight, pH, volatile fatty acids (VFA) and lactic acid concentrations were determined.

The remaining rats were fed on diets containing increasing amounts of fermentable

carbohydrates. Each diet was given for 3 weeks. Thus, eighteen rats were fed on the diet containing 40 g lactose/kg. At the end of a 3-week period, faeces were collected for 3 d for bile acid analysis. As the bile acid composition of faeces was not different from that of the control group, no rat was killed and they received the diet containing 80 g lactose/kg for a further 3 weeks. The bile acid composition of faeces remained unchanged and rats then received the diet containing 160 g lactose/kg for another 3-week period. At the end of this period, as the bile acid composition of faeces was modified, a group of six rats was killed (one rat every 4 h for 24 h). The remaining twelve rats were fed on 320 g lactose/kg diet for 3 weeks and then a group of rats killed. The remaining six rats were fed on 480 g lactose/kg diet for a 3-week period and then killed.

The same procedure was used for lactulose. Rats were fed successively on diets containing 40, then 80 and 160 g lactulose/kg diet. With this carbohydrate, whatever the level of incorporation, the bile acid composition of faeces was different from that of the control group. A group of six rats was killed at the end of each dietary treatment. The same procedure was used also for amylomaize and potato-starch diets (160, 320 and 480 g/kg).

Expt 2. For each carbohydrate, a group of four rats received the control diet for an initial period of 2 weeks and then each of the different diets up to the highest level of poorly digestible carbohydrate intake. As described previously, each diet was given for 3 weeks, then rats were introduced into plastic sterilized isolators. After 3 d, feeding of poorly digestible carbohydrates was stopped and rats were fed on the control diet for 32 d.

Faeces were collected for bile acid analyses, first at the end of the initial period, second just before the fermentable carbohydrate feeding was stopped, and finally between days 4 and 6, 16 and 18, and 24 and 31 after changing to the control diet. On day 32, rats were killed at 14.00 hours and caecal pH, VFA and lactic acid concentrations were determined. A control group of four rats (C2) was fed on the maize-starch diet for 24 weeks and killed at 14.00 hours to compare caecal pH, VFA and lactic acid concentrations with those of rats receiving fermentable carbohydrates.

Analysis

Amount of carbohydrates fermented in the intestine. At 5 d before death, a transit marker, [^{14}C]polyethylene glycol, molecular weight 4000, was incorporated into the diet. When marker excretion was similar to ingestion, rats were killed every 4 h for 24 h. The contents of the last 50 mm of the ileum and colon were collected, washed out and the relative concentrations of carbohydrate and marker determined. The ratio, R, of carbohydrate concentration: [^{14}C]polyethylene glycol concentration was measured in the contents and in diets. From all R values obtained for a 24 h period it was possible to determine the relative amount of carbohydrate fermented by the large-intestine microflora (Cf):

$$\mathrm{Cf}(\%) = \frac{R\,\text{ileum} - R\,\text{colon}}{R\,\text{in diet}} \times 100.$$

An estimation of levels of daily carbohydrate fermented was given by multiplying Cf by daily carbohydrate intake.

Lactose and lactulose. The levels were determined by an enzymic method (Boehringer, Mannheim) with β-galactosidase (*EC* 3.2.1.23) and β-galactose dehydrogenase (*EC* 1.1.1.48).

Starches. For diets and faeces, starches were dispersed in dimethylsulphoxide (800 ml/l) by autoclaving for 20 min. They were then determined enzymically with amyloglucosidase (*EC* 3.2.1.3) (Optidex 1200; Roquette frères, Lestrem) and glucose oxidase (*EC* 1.1.3.4) (Biotrol, Paris).

Lactic acid. The levels were determined enzymically with lactate dehydrogenase (*EC* 1.1.1.27) (Boehringer, Mannheim).

VFA. The levels were determined using gas–liquid chromatography (Ottenstein & Bartley, 1971).

Bacterial transformation of bile acids. Cholic acid was obtained from Stera loids Inc. (Pawling, New York) and [24−^{14}C]cholic acid from CEA (Saclay), [24−^{14}C]β-muricholic acid was prepared from axenic rats, as described previously (Sacquet *et al.* 1985).

In vitro bacterial transformations of bile acid were determined as follows: 200 μg [24−^{14}C]cholic acid and 0·1 g 1·4-dithiotreitol/l were mixed with 5 ml faecal suspension diluted tenfold from control rats (Cl) in 0·2 M-citric acid or phosphate buffer (pH 4·5–7·0). These diluted samples were incubated in an anaerobic Freter chamber, at 37° in hydrogen–nitrogen (10:90, v/v). After incubation, samples were acidified to pH 2·0 and extracted with chloroform–methanol (2:1, v/v). After evaporation of chloroform, bile acids were diluted in methanol, methylated with diazomethane and separated by thin-layer chromatography on silica gel G with a mobile phase of chloroform–acetone–methanol (70:25:5, by vol.). The radioactivity in labelled bile acid methyl esters was measured using a radio scanner (Berthold).

The in vivo bacterial transformations of bile acids were determined from faecal bile acid composition. After hot ethanol extraction in a Kumagawa apparatus for 48 h, neutral and acid sterols were separated by the method of Grundy *et al.* (1965). Bile acids were methylated with diazomethane and determined by gas–liquid chromatography as trimethyl silyl derivatives using a 25 m OV 1701 capillary column.

Faecal bile acid composition was used to determine the major bacterial transformations of bile acids.

The results are expressed as the percentage cholic acid transformed to deoxycholic acid and percentage β-muricholic acid transformed to hyodeoxycholic acid or to ω-muricholic acid.

Statistical methods

Statistical analysis (Snedecor & Cochran, 1967) involved variance analysis and Newman Keuls's multiple range test, when the Bartlett test showed equality of variance. For non-parametric values, the Mann-Whitney U test was used for independent samples (Expt 1) and Wilcoxon rank test for related samples (Expt 2). Pairwise comparisons were restricted to two comparisons for each experiment: one to the control group and one to the lowest level of carbohydrate incorporation in Expt 1, one to the control group and one to the highest level of carbohydrate incorporation in Expt 2. The statistical significances of correlation coefficients were determined using the Spearman test (Lindley & Scott, 1984).

RESULTS

Carbohydrate digestion

Varying percentages of digestible carbohydrates escaped enzymic digestion in the small intestine: maize starch 3, lactose 35–50, amylomaize starch 35–40, potato starch 76–77, lactulose 100. In the large intestine, all lactose and lactulose reaching the caecum were degraded by the intestinal microflora, whereas 4–10% of ingested amylomaize starch and 16–25% of ingested potato starch were not hydrolysed and were excreted. Table 1 shows the daily amounts of ingested carbohydrates reaching the caecum and fermented in the large intestine.

Changes in the caecum

Expt 1. Fermentation of the different carbohydrates increased the caecal weight (Table 2). A highly significant correlation (r 0·997, $P < 0·0001$) was observed between caecal weight

Table 1. *Expt 1. Amounts of ingested carbohydrate reaching the caecum and fermented in the large intestine of rats fed on different levels of poorly digestible carbohydrates*

(Values are means with their standard errors for six rats)

		Amounts of carbohydrate (g/d)					
		Intake		Reaching the caecum		Fermented	
Carbohydrate	(g/kg diet)	Mean	SEM	Mean	SEM	Mean	SEM
Maize starch	580	8·93[a]	0·41	0·26[j]	0·10	0·23[j]	0·01
Lactose	160	2·34[f]	0·06	0·83[h]	0·12	0·83[h]	0·12
	320	4·85[d]	0·12	2·20[g]	0·30	2·20[g]	0·20
	480	5·77[c]	0·14	2·89[f]	0·36	2·73[f]	0·32
Lactulose	40	0·53[i]	0·02	0·52[i]	0·02	0·52[i]	0·02
	80	1·05[h]	0·01	1·00[h]	0·05	1·00[h]	0·05
	160	2·00[g]	0·04	1·98[g]	0·08	1·98[g]	0·08
Amylomaize starch	160	2·26[fg]	0·09	0·78[hi]	0·17	0·59[i]	0·08
	320	4·50[d]	0·10	1·85[g]	0·11	1·39[h]	0·16
	480	4·42[b]	0·18	2·90[f]	0·28	2·51[f]	0·40
Potato starch	160	2·38[f]	0·15	1·81[g]	0·14	1·21[h]	0·12
	320	4·99[d]	0·04	3·76[e]	0·27	2·72[f]	0·12
	480	7·49[b]	0·16	5·76[c]	0·40	4·52[d]	0·16

[a–j] Values with different superscript letters were significantly different (Newman Keuls's test) ($P < 0·05$).

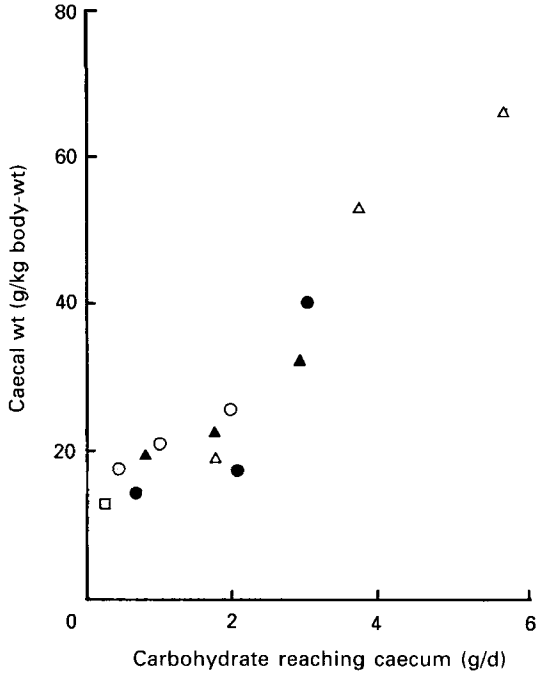

Fig. 2. Expt 1. Variations in caecal weight and amounts of the different poorly digestible carbohydrates reaching the caeca of rats. (□), Maize-starch; (●), lactose; (○), lactulose; (▲), amylomaize starch; (△), potato starch. For details of diets, see p. 104 (r 0·96, $P < 0·0001$).

Table 2. *Expt 1. Effect of different levels of poorly digestible carbohydrates on caecal weight, water content and pH of rats*

(Values are means with their standard errors for six rats)

Carbohydrates	(g/kg diet)	Caecal wt (% of body-wt) Mean	SEM	Caecal water content (mg/g) Mean	SEM	pH Mean	SEM
Maize starch	580	1·29	0·05	762	14	6·7	0·05
Lactose	160	1·52	0·02	760	5	6·4*	0·1
	320	2·37*†	0·10	809*	27	6·0*†	0·2
	480	4·1*†	0·20	816*†	16	5·8*†	0·2
Lactulose	40	1·84*	0·10	772	5	6·1*	0·05
	80	2·16*	0·11	767	5	6·0*	0·1
	160	2·64*†	0·11	777	4	5·9*	0·1
Amylomaize starch	160	1·88*	0·10	770	7	6·2*	0·17
	320	2·3*	0·11	752	6	5·9*†	0·13
	480	3·4*†	0·15	756	8	5·9*†	0·1
Potato starch	160	2·65*	0·10	714*	14	6·1*	0·1
	320	5·40*†	0·30	729*	11	5·6*†	0·1
	480	6·60*†	0·30	743	23	5·3*†	0·2

The individual values from rats killed at the same hour of the day were compared using Mann-Whitney U test ($P < 0·05$):

* Significantly different from the maize starch diet.

† Significantly different from the lowest level of the same carbohydrate in the diet.

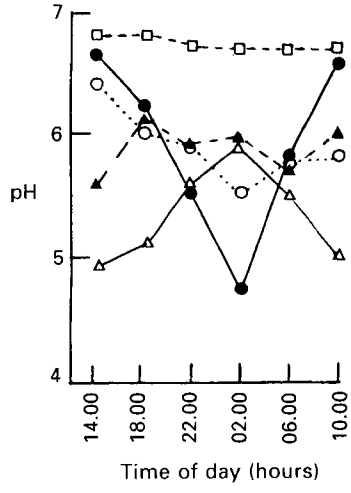

Fig. 3. Expt 1. Variations in caecal pH during a 24 h period in rats fed on the highest levels of poorly digestible carbohydrates. (□—□), Maize-starch diet; (●—●), 480 g lactose/kg diet; (○···○), 160 g lactulose/kg diet; (▲—▲), 480 g amylomaize starch/kg diet; (△—△), 480 g potato starch/kg diet. For details of diets, see p. 104.

and the amount of carbohydrate entering the caecum (Fig. 2), whatever the ingested carbohydrate.

Caecal water content increased with increasing levels of lactose in the diet. It was unchanged with lactulose and amylomaize starch and decreased with potato starch (Table 2).

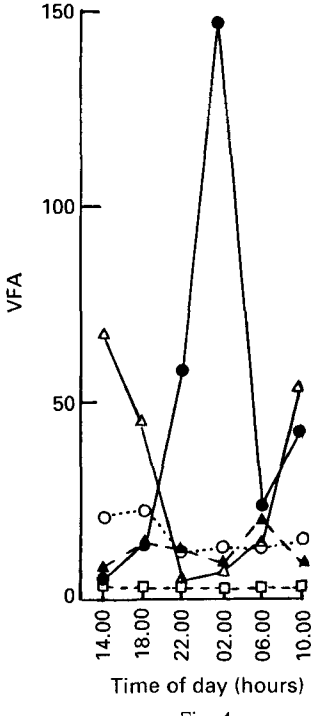

Fig. 4.

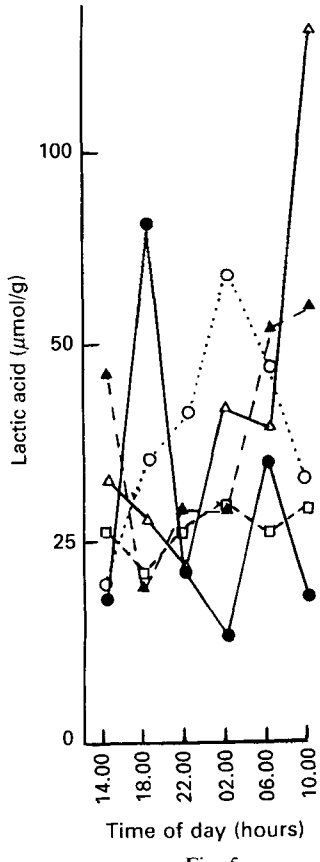

Fig. 5.

Fig. 4. Expt 1. Variations in caecal volatile fatty acid (VFA) concentrations during a 24 h period in rats fed on the highest levels of poorly digestible carbohydrates. (□--□), Maize-starch diet; (●—●), 480 g lactose/kg diet; (○···○), 160 g lactulose/kg diet; (▲--▲), 480 g amylomaize starch/kg diet; (△—△), 480 g potato starch/kg diet. For detail of diets, see p. 104.

Fig. 5. Expt 1. Variations in caecal lactic acid concentrations during a 24 h period in rats fed on the highest levels of poorly digestible carbohydrates. (□--□), Maize-starch diet; (●—●), 480 g lactose/kg diet; (○···○), 160 g lactulose/kg diet; (▲--▲), 480 g amylomaize starch/kg diet; (△-△), 480 g potato starch/kg diet. For details of diets, see p. 104.

Caecal pH exhibited large variations during the 24 h period according to the nature and dietary levels of carbohydrates. Fig. 3 shows caecal pH for rats fed on the highest levels of carbohydrates. The minimum pH did not occur at the same time of the day for different diets and the pH variations were different for each carbohydrate. pH variations were larger with lactose and potato starch than with lactulose and amylomaize starch. For rats killed at the same time of day, pH was lower in rats ingesting fermentable carbohydrates than in control rats (Mann-Whitney U test). It varied significantly with the levels of carbohydrates in the diet in all cases except for lactulose (Table 2).

Some changes in caecal VFA and lactic acid concentrations in rats fed on the highest levels of poorly digestible carbohydrates are shown in Figs 4 and 5. As for pH values, the highest VFA and lactic acid concentration did not occur at the same time of day for these two diets. Table 3 shows the mean caecal VFA and lactic acid concentrations for all diets.

Table 3. *Expt 1. Lactic acid and volatile fatty acid (VFA) concentrations (μmol/g) in caeca of rats fed on different levels of poorly digestible carbohydrates*

(Values are means with their standard errors for six rats)

		Lactic acid		Total VFA		Acetate		Propionate		Butyrate		Isovalerate		Valerate	
Carbohydrate	(g/kg diet)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Maize starch	580	1·5	2	54	2	32·9	1·3	15·1	0·6	3·7	0·3	1·4	0·2	0·9	0·2
Lactose	160	8*	4	52	7	24·7*	2·2	14·0	2·5	10*	2	0·7	0·6	0·9	0·1
	320	48*†	22	45	10	30·6	7·1	10·4	3·5	3·3†	0·8	0·4*	0·1	0*†	
	480	49*†	20	58	16	37	10	12·9	3·0	3·7†	1·6	0·4*	1·6	0*†	
Lactulose	40	3*	1	59	8	38·1*	0·5	12·4	1·7	7*	1	0·5*	0·1	1·3	0·2
	80	3*	0·5	68*	5	27·2†	1·6	38*†	2	2·4†	0·3	0·7*	0·2	0*†	
	160	16*†	2	78*	10	41·4	2·5	32·0*†	0·6	2·5†	0·3	0·6*	0·1	0*†	
Amylomaize starch	160	14*	2	90*	16	54*	7	31*	7	3·7	0·3	1·2	0·3	0·3*	0·1
	320	8*†	1	71	10	34†	4	32*	5	4·6	0·4	0·6*	0·1	0·3*	0·1
	480	12*	2	75	10	40	3	45·2*	2·5	7·3*†	1·9	0·4*	0·2	0*†	
Potato starch	160	2·5	0·6	52	4	34	2	14·1	1·8	2·5	0·3	0·4*	0·1	1·6	0·1
	320	3	1	50	7	35·7	0·6	10·5	0·9	3·0	0·3	0·9	0·2	0·1*†	0·1
	480	33*†	10	88*	21	66·0*†	15	14·2	5·1	6·9*†	1·6	1	0·6	0*†	

The individual values from rats killed at the same hour of the day were compared using Mann-Whitney U test ($P < 0·05$).

* Significantly different from the maize starch diet.

† Significantly different from the lowest level of the same carbohydrate in the diet.

Table 4. *Expt 2. Caecal weight, pH, and lactic acid and volatile fatty acid (VFA) concentrations on day 32 after the interruption of fermentable carbohydrate ingestion*

(Values are means with their standard errors for four rats)

Dietary carbohydrate...		Maize starch*	Lactose†	Lactulose†	Amylomaize starch†	Potato starch†
Caecum wt (% of body-wt)	Mean	1·55[a]	1·83[b]	1·56[a]	1·6[a]	1·52[a]
	SEM	0·05	0·05	0·02	0·1	0·1
pH	Mean	6·7[c]	6·37[a]	6·55[b]	6·62[c]	6·63[c]
	SEM	0·04	0·04	0·03	0·02	0·05
Lactic acid (μmol/g)	Mean	1·9[a]	1·7[a]	2·1[a]	2·3[a]	2·9[b]
	SEM	0·5	0·2	0·6	0·3	0·4
Total VFA (μmol/g)	Mean	40[b]	36[b]	29[b]	34[b]	23[a]
	SEM	4	3	1	2	2
Acetate (μmol/g)	Mean	24·6[c]	21·6[b]	16·8[a]	21·4[b]	15·0[a]
	SEM	0·3	0·4	0·5	0·3	0·5
Propionate (μmol/g)	Mean	10·7[c]	10·3[c]	8·6[b]	8·8[b]	5·6[a]
	SEM	0·1	0·1	0·2	0·1	0·2
Butyrate (μmol/g)	Mean	3·1[c]	3·4[c]	2·6[b]	1·2[a]	2·0[b]
	SEM	0·1	0·2	0·2	0·1	0·1
Isovalerate (μmol/g)	Mean	0·8[b]	0·7[b]	0·6[b]	0·3[a]	0·4[a]
	SEM	0·04	0·03	0·03	0·1	0·1
Valerate (μmol/g)	Mean	0·9[b]	0[a]	0[a]	0·7[b]	0[a]
	SEM	0·1			0·1	

[a–c] Values in the same horizontal row with different superscript letters were significantly different (Newman–Keuls's multiple range test): $P < 0·05$.

* Control group C2.

† Diet ingested before the interruption of fermentable carbohydrate ingestion.

In some groups the standard deviation of the mean was high because variations in caecal VFA concentration during the 24 h period increased. However, whatever the time of killing, the effect of the diet on caecal lactic acid concentration was significant ($P < 0·05$; Mann-Whitney U test) for all diets except for potato starch (160 and 320 g/kg). By contrast, poorly digestible carbohydrates did not significantly modify the caecal VFA concentration, with the exception of the lactulose diet (80 and 160 g/kg), amylomaize starch (160 g/kg) and potato starch (480 g/kg). There was no correlation between caecal lactic acid or VFA concentration and the level of dietary carbohydrate.

The proportion of poorly digestible carbohydrates in the diet and the nature of these carbohydrates influenced the composition of the organic acids produced by caecal fermentation (Table 3). Compared with rats fed on maize starch, the acetate concentration was lower in the caeca of rats fed on 160 g lactose/kg and 80 g lactulose/kg diets and higher in rats fed at the highest level of potato starch. The propionate concentration was increased in the caeca of rats fed on 80 and 160 g lactulose or amylomaize starch/kg diets. Propionate remained unchanged in rats fed on potato-starch diets. The butyrate concentration was higher in rats fed on 160 g lactose/kg and 40 g lactulose/kg diets, corresponding to the lowest level of incorporation, and in rats fed on amylomaize diets or 480 g potato starch/kg diet, the highest level of incorporation. Caecal concentrations of isovalerate and valerate decreased in most cases, and valerate totally disappeared from the caecum of rats fed on the highest levels of carbohydrates.

Expt 2. After the interruption of fermentable carbohydrate ingestion (Table 4), values for caecal weight and pH of rats fed on lactose diets were slightly lower than those of other rats,

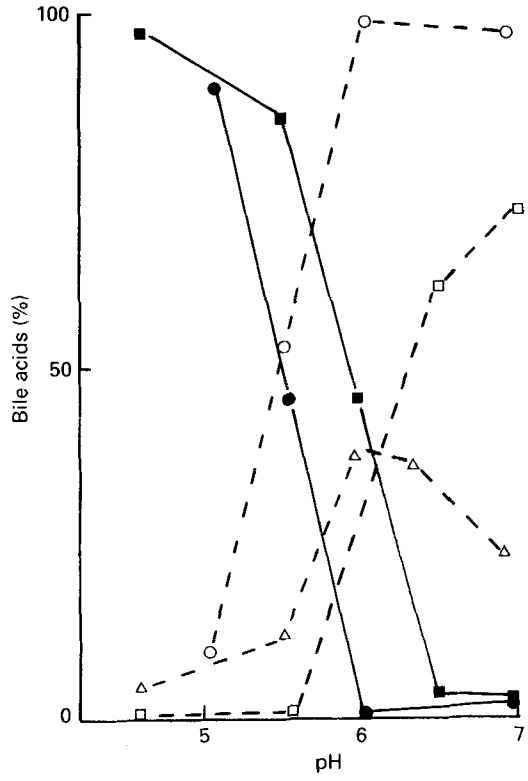

Fig. 6. Percentage bacterial transformation of cholic acid and β-muricholic acid in vitro relative to pH. (●—●), Cholic acid; (○--○), deoxycholic acid; (■—■), β-muricholic acid; (□--□), hyodeoxycholic acid; (△--△), ω-muricholic acid. For details of these transformations, see Fig. 1, p. 104.

and valerate, which was absent in all the caeca, did not reappear except in rats fed on amylomaize diets.

Bacterial transformations of bile acids

In vitro transformations of bile acids (*Fig. 6*). At pH 7·0, cholic acid was totally transformed into deoxycholic acid; 74% of β-muricholic acid was converted to hyodeoxycholic acid and 19% to ω-muricholic acid. Between pH 7·0 and pH 6·0, hyodeoxycholic acid formation was reduced and that of ω-muricholic acid increased; cholic acid transformation was not affected. At pH 5·5, the percentage of β-muricholic acid increased because hyodeoxycholic acid was not formed and ω-muricholic acid formation was greatly decreased. At this pH cholic acid transformation was 50% lower. At pH 5·0 less than 10% of β-muricholic acid and cholic acid were transformed.

In vivo transformations of bile acids (*Table* 5): *Expt 1.* In rats fed on maize starch, the microbial flora converted all cholic acid to deoxycholic acid and almost all β-muricholic acid to hyodeoxycholic acid. Ingestion of increasing amounts of fermentable carbohydrates gradually modified the bacterial transformations. Some of the changes were similar for each carbohydrate. The first change was a decrease in the transformation of β-muricholic acid into hyodeoxycholic acid, while the proportion of ω-muricholic acid increased. The second change was a decrease in cholic acid transformation to deoxycholic acid. Thus, in the faeces of rats fed on 320 g lactose/kg, 80 g lactulose/kg or 480 g potato starch/kg diets, hyodeoxycholic acid almost disappeared when 50% of cholic acid was transformed into

Table 5. *Expt 1. Bacterial transformations of bile acids in rats fed on different levels of poorly digestible carbohydrates*
(Values are means with their standard errors for six rats)

Carbohydrate	(g/kg diet)	Cholic acid transformation to deoxycholic acid (%)		β-Muricholic acid transformation (%) to: Hyodeoxycholic acid		β-Muricholic acid transformation (%) to: ω-Muricholic acid		β-Muricholic acid untransformed (%)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Maize starch	580	100	0	90	2	1	1	9	2
Lactose	40	99	1	86	5	3	2	11	5
	80	98	1	81	2	3	2	16	2
	160	87*	6	68	15	11*	7	21	11
	320	44*†	14	0*†		21*	7	79*†	7
	480	31*†	12	2*†	2	9*	6	88*†	2
Lactulose	40	80*	8	36*	17	26*	9	38*	10
	80	49*†	19	0*†		26*	4	74*†	4
	160	34*†	19	0*†		32*	8	68*†	8
Amylomaize starch	160	98	2	63		16*	8	20	6
	320	90	2	7*†	7	31*†	5	62*†	7
	480	80*†	2	0*†		56*†	7	44*†	7
Potato starch	160	78	7	34*	5	11*	1	34*	4
	320	74	9	13*†	11	26*	11	61*†	12
	480	39*†	13	1*†	1	12*	8	87*†	8

Comparisons between pairs of groups were made by the Mann-Whitney U test ($P < 0.05$).
* Significantly different from the maize starch diet.
† Significantly different from the lowest level of the same carbohydrate in the diet.

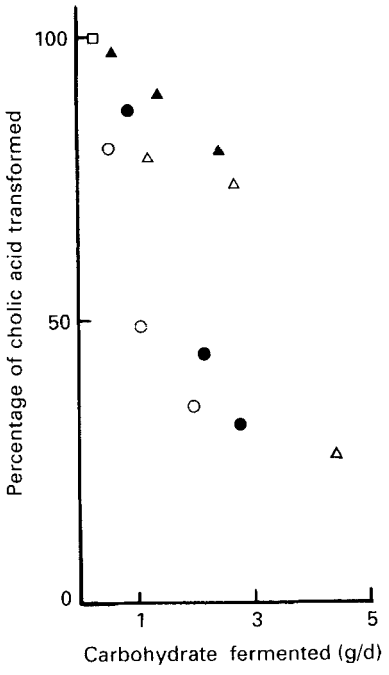

Fig. 7.

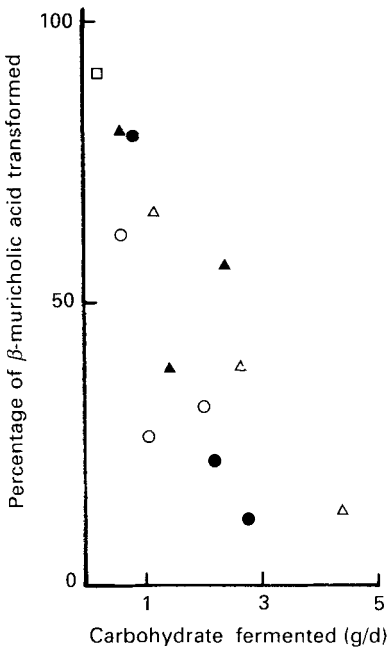

Fig. 8.

Fig. 7. Expt 1. Percentage of cholic acid transformed in vivo relative to the amounts of carbohydrate fermented by rats fed on different levels of poorly digestible carbohydrates. (□), Maize-starch diet; (●), lactose diets; (○), lactulose diets; (▲), amylomaize-starch diets; (△), potato-starch diets. For details of diets see p. 104 (r −0·725, $P < 0{\cdot}005$).

Fig. 8. Expt 1. Percentage of β-muricholic acid transformed in vivo relative to the amounts of carbohydrate fermented by rats fed on different levels of poorly digestible carbohydrates. (□), Maize-starch diet; (●), lactose diets; (○), lactulose diets; (▲), amylomaize-starch diets; (△), potato-starch diets. For details of diets, see p. 104 (r −0·75, $P < 0{\cdot}003$).

deoxycholic acid. However, for some carbohydrates modifications differed. Thus, in rats fed on amylomaize starch, the percentage of deoxycholic acid remained high. When the percentage of hyodeoxycholic acid was lowest, bacterial transformation of β-muricholic acid to ω-muricholic acid tended to decrease in the faeces of rats fed on lactose and potato starch.

Despite the individual variability, a significant negative correlation was found between the amounts of fermented carbohydrate and the percentages of cholic and β-muricholic acids transformed by the microflora; correlation coefficients were: cholic acid 0·725 ($P < 0{\cdot}005$), β-muricholic acid 0·75 ($P < 0{\cdot}003$) (Figs 7 and 8). No correlation was found between the mean caecal pH observed for a 24 h period and bacterial transformation of bile acids. By contrast, a significant positive correlation was found between the minimum caecal pH and the bacterial transformation of cholic and β-muricholic acids. In these cases, the correlation coefficients were: cholic acid 0·81 ($P < 0{\cdot}001$), β-muricholic acid 0·88 ($P < 0{\cdot}0001$) (Figs 9 and 10).

No correlation was found between bile acid transformations and VFA or lactic acid caecal concentrations.

Expt 2. Table 6 shows the variation in bile acid transformation in the same rats during

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Table 6. *Expt 2. Changes in bacterial transformations of bile acids in rats fed on maize starch during the initial period, then increasing levels of poorly digestible carbohydrates up to the highest level and the maize-starch diet after isolation of the rat*

(Values are means with their standard errors for four rats, except where indicated in parentheses)

Dietary carbohydrate	Period of feeding (d)	Cholic acid transformation to deoxycholic acid (%)				β-Muricholic acid transformation (%) to:				β-Muricholic acid untransformed (%)	
		Mean		SEM		Hyodeoxycholic acid		ω-Muricholic acid			
						Mean	SEM	Mean	SEM	Mean	SEM
Maize starch		97		3		87	5	3	2	10	3
Lactose		31*		2		0*	0	18	9	82*	9
Maize starch	4–6	50*†(2)	0*†(2)	3	0	2*	1	54*	12	44*	10
	15–17	71*†(2)	0*†(2)	2	0	0*	0	71*†	9	29†	9
	29–31	72*†(2)	0*†(2)	3	0	0*	0	79*†	8	21†	7
Maize starch		100		0		96	2	3	2	1	0
Lactulose		21*		7		0*	0	29*	8	71*	8
Maize starch	4–6	63*†(2)	0*†(2)	1	0	0*	0	92*†	3	8†	3
	15–17	77*†(2)	0*†(2)	3	0	0*	0	90*†	2	10†	2
	28–31	85*†(2)	0*†(2)	1	0	0*	0	87*†	6	13*†	6
Maize starch		95		4		96	3	2	1	2	1
Amylomaize		77*		3		1*	0	50*	12	49*	11
Maize starch	4–6	85		3		1*	0	84*	3	15†	2
	15–17	74*		2		0*	0	89*†	2	11†	2
	29–31	83*		2		0*	0	90*†	1	10†	1
Maize starch		95		3		86	1	0	0	14	1
Potato starch		71*		6		0*	0	0	0	100*	1
Maize starch	4–6	84*		1		2*	1	88*†	4	10†	4
	15–17	79*		3		0*	0	87*†	4	13†	4
	29–31	71*		3		0*	0	82*†	5	18†	5

Statistical analysis compared the same rats in different dietary conditions (Wilcoxon rank test, $P < 0{\cdot}06$).

* Significantly different from the initial period of maize-starch feeding.

† Significantly different from the highest level of carbohydrate feeding.

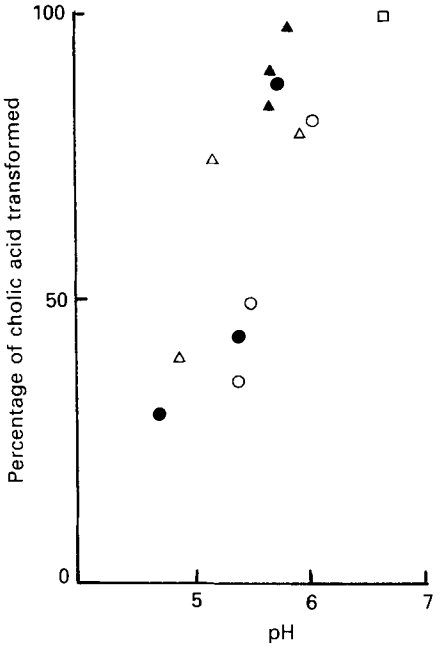

Fig. 9.

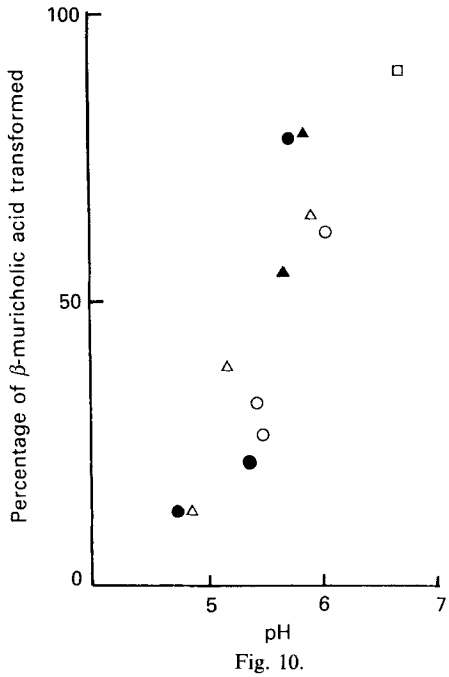

Fig. 10.

Fig. 9. Expt 1. Percentage of cholic acid transformed in vivo relative to minimum caecal pH of rats fed on different levels of poorly digestible carbohydrates. (□), Maize-starch diet; (●), lactose diets; (○), lactulose diets; (▲), amylomaize-starch diets; (△), potato starch diets. For details of diets, see p. 104 (r 0·81, $P < 0·001$).

Fig. 10. Expt 1. Percentage of β-muricholic acid transformed in vivo relative to minimum caecal pH of rats fed on different levels of poorly digestible carbohydrates. (□), Maize-starch diet; (●), lactose diets; (○), lactulose diets; (▲), amylomaize-starch diets; (□), potato-starch diets. For details of diets, see p. 104 (r 0·88, $P < 0·0001$).

the initial period of maize-starch feeding, the ingestion of the highest level of carbohydrates and the refeeding of maize starch. As in Expt 1, changes in bile acid transformations were observed in all rats fed on the highest levels of poorly digestible carbohydrates. Transformation of cholic acid into deoxycholic acid was less affected by amylomaize and potato starches than by lactose and lactulose. After carbohydrate ingestion was stopped, the transformation of cholic acid was unchanged in rats fed on amylomaize or potato starch. In those fed on lactose or lactulose, cholic acid transformation totally disappeared in two rats of each group, and increased in the other two, but was lower than it had been initially. The bacterial transformation of β-muricholic acid into hyodeoxycholic acid was not re-established. On the contrary, bacterial transformation of β-muricholic acid into ω-muricholic acid was re-established rapidly, even when it had totally disappeared, and it became as important as the initial transformation of β-muricholic acid into hyodeoxycholic acid.

DISCUSSION

Lactose, lactulose, amylomaize and potato starches were digested differently in the small intestine before entering the caecum. The increase in the caecal volume seems to be more dependent on the amount of indigestible carbohydrate than on VFA production. The

activity of the digestive enzymes is low in the caeca of conventional rats (Reddy *et al.* 1969) and most undigested carbohydrates are fermented by the microbial flora.

Fermentations were characterized by a large variability: there were large circadian variations in caecal pH related to the ingested carbohydrate, a large pH difference between the different carbohydrates for the same amount of fermented carbohydrate, and a large difference in the nature of fermentation products related to the carbohydrate and to the amounts of ingested carbohydrates.

There were also large individual variations in the bacterial enzyme activities involved in bile acid transformations. However, the variation in bacterial transformations always occurred in the same way.

Irrespective of the carbohydrate, hyodeoxycholic acid disappeared first in favour of ω-muricholic acid before the transformation of cholic into deoxycholic acid decreased. No correlation was found between bile acid transformations and VFA or lactic acid concentrations. With similar bile acid transformations, large differences were observed in the concentrations of VFA or lactic acid. Thus, fermentation of increasing amounts of lactose or lactulose affected the transformation of cholic acid into deoxycholic acid. Fermentation of lactose increased caecal lactic acid concentration, while that of lactulose increased caecal VFA concentration. Transformation was not related to the caecal concentration of some VFA. In contrast, a decrease in intestinal pH seemed to play a major role in the observed effects. The correlation between transformation and caecal pH was significant when the minimum pH observed in each group was taken into account rather than the mean pH which was less variable. This suggested that the bacterial transformations of bile acids mainly occurred when the fermentations were more active in the 24 h period.

The large similarity between the effect of pH in vitro and in vivo suggested that the effects of poorly digestible carbohydrates resulted from the decrease in caecal pH and from an inhibition of enzyme activities whose maximum pH is almost neutral (Aries & Hill, 1970*a*, *b*). However, in vivo, some important effects were observed which were related to the nature of the carbohydrate and were independent of pH: (1) lactulose modified the bacterial transformations of bile acids more rapidly and lowered the intestinal pH less than the other carbohydrates; (2) amylomaize-starch fermentation had little effect on the transformation of cholic acid into deoxycholic acid, although the pH was modified; (3) the transformation of β-muricholic into ω-muricholic acid was more important with amylomaize starch than with the other carbohydrates, although the caecal pH was not lowered more by this carbohydrate than by others.

When carbohydrate ingestion was stopped, the caecal pH returned to its initial value (6·7). This was not the case for bacterial enzyme activities. This indicates that pH was not the only factor responsible for the observed effects, and that most probably the bacterial population itself was modified. Hyodeoxycholic acid, which had totally disappeared in rats ingesting the higher levels of carbohydrate, was not found, probably because the bacteria responsible for this transformation had been replaced by bacteria converting β-muricholic acid into ω-muricholic acid. A very large individual variability was noticed in cholic acid transformation. This was illustrated by the fact that in half the rats ingesting high amounts of lactose and lactulose, cholic acid was not transformed into deoxycholic acid, while in the other half this transformation was partly restored. These variations do not seem to be related to the pH or to the composition of fermentation products because it was not observed with poorly digestible starches. Further studies should be made to explain why certain transformations are restored in some rats and disappear in others.

Our findings indicate the high instability of microbial flora and provide evidence for the modification of this flora as affected by the ingestion of poorly digestible carbohydrates.

They also suggest that poorly digestible carbohydrates modify bacterial transformations by at least two mechanisms: inhibition of the bacterial enzyme activities dependent on pH and modification of the microbial flora.

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