

Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat

BY HASSAN YOUNES, CHRISTIAN DEMIGNÉ* AND CHRISTIAN RÉMÉSY

*Laboratoire des Maladies Métaboliques, INRA de Clermont-Ferrand/Theix, F-63122
Saint-Genès-Champanelle, France*

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The effect of fermentation on colonic absorption of Ca and Mg was investigated in 8-week-old rats adapted to diets containing either digestible wheat starch (DS diets) or including resistant starch, i.e. 350 g raw potato starch/kg (RS diets). The dietary Ca level of the DS and RS diets was 2.5 or 7.5 g/kg. RS diets resulted in enlargements of the caecum together with hypertrophy of the caecal wall. Acidification of the caecal contents by microbial fermentation of RS was influenced by the dietary Ca level. Very acidic pH conditions and relatively low concentrations of short-chain fatty acids, in the presence of lactic acid fermentation, were observed with the 2.5 g Ca/kg level. Rats fed on RS diets had a higher percentage of soluble Ca (and inorganic phosphate) in the caecum, particularly of rats adapted to the high Ca level. As a result of the hypertrophy of the caecal wall and of an elevated concentration of soluble Ca, the caecal absorption of Ca was 5–6-fold higher in the RS groups than in the DS groups. The difference between dietary intake and faecal excretion (DI–FE) of Ca was higher in rats fed on RS diets than in those fed on DS diets, when the dietary Ca level was 2.5 g/kg. With the higher Ca intake the elevated rate of Ca absorption from the caecum in RS-fed rats was not paralleled by an enhanced DI–EE difference: this suggests a shift of the Ca absorption towards the large intestine. Feeding RS diets also enhanced Mg caecal absorption, resulting in a substantially higher DI–FE difference for Mg, especially with the 2.5 g Ca/kg diets, because a high Ca intake tends to inhibit Mg absorption. The present findings support the view that the large intestine may represent a major site of Ca (and Mg) absorption when acidic fermentations take place. This process could improve the digestive Ca balance when the dietary Ca supply is low; when the Ca supply is affluent, it rather shifts Ca absorption towards a more distal site of the digestive tract.

Calcium: Resistant starch: Caecum: Fermentation: Rat

The effect of large intestinal fermentation on the bioavailability of minerals, especially Ca, is still uncertain. The lumen pH, the caecal surface area and the concentration of organic acids in the large intestine are closely associated with the quantities of fermentable carbohydrates, as well as mineral concentrations (Demigné *et al.* 1989). Thus, in contrast with the small intestine, it is necessary to take microbial fermentation into account when investigating the mechanisms governing the availability and the possible absorption of minerals by the colon. Dietary fibre or resistant starch (RS) represent a major source of complex carbohydrates for the intestinal microflora in animals or humans (McFarlane & Cummings, 1991). Carbohydrates that escape digestion in the small intestine are substrates for the formation of short-chain fatty acids (SCFA) in the large intestine, which allows the recovery of part of their energy (Morand *et al.* 1992). Under certain circumstances, foods rich in complex carbohydrates (for example, wheat bran) may alter the digestive balance of essential minerals, by impairing absorption in the small intestine (Rheinhold *et al.* 1976; Donagelo & Eggum, 1986). However, minerals strongly associated with plant cell walls can

* For reprints.

be released only by the microbial breakdown of these complex polysaccharides in the large intestine. Thus, it is important to consider the contribution of the colon in the overall absorption of minerals, especially in relation to the major divalent cations. In fact, frequently the apparent absorption of minerals is unchanged by dietary fibre, but fibre may shift the major site of absorption towards the large intestine (Cummings *et al.* 1979; Demigné *et al.* 1989). It has been shown that the large intestine is a major site of Mg absorption (Hardwick *et al.* 1990; Lutz *et al.* 1991), which is consistent with the fact that Mg (mainly present in plant foods) becomes fully available after the microbial digestion of fibre. Efficient absorption of Ca in the rat caecum is possible, but the contribution of the large intestine is still unclear (Petith & Schedl, 1976; Hylander *et al.* 1980; Nellans & Goldsmith, 1981; Schulz *et al.* 1993).

Thus, it appeared interesting to consider the mechanisms by which fermentation could influence the absorption of Ca and Mg. Previous work has shown a positive effect of complex carbohydrates and oligosaccharides on Ca digestibility, probably by improving the solubility of this cation in caecal contents (Levrat *et al.* 1991 *a, b*; Rémésy *et al.* 1993). Studies on the interactions between Ca and fermentation are relevant also because minerals, particularly Ca, are essential to the equilibrium established between the host and the microflora and they have probably a protective effect on the colonic mucosa against cytotoxic agents (Lipkin, 1991). This may reflect the fact that a high dietary Ca intake drastically depresses the solubility of fatty acids as well as that of bile acids in the large intestine (Gorvers & Van der Meer, 1993).

The present investigation examined the role of fermentation on the distal absorption of divalent cations and the consequences on their digestive balance. The present study also sought to clarify the reciprocal interactions between Ca absorption in the upper and lower parts of the digestive tract, and the effect of dietary Ca intake on microbial fermentations in the large intestine.

MATERIALS AND METHODS

Animals and diets

Forty male Wistar rats (IFA-CREDO, L'Arbresle, France) were fed on a commercial pelleted diet (UAR, Villemoisson s/Orge, France) until their body weights reached approximately 200 g (8 weeks). Groups of ten rats were fed for 21 d on a basal (fibre-free; DS) purified diet, or diets containing 350 g RS/kg (Table 1). RS was a raw potato starch supplied by Louis François, St-Maur, France; about 750 g/kg of this starch was amylase (EC 3.2.1.1)-resistant (Andrieux *et al.* 1989). The animals were housed two per cage (wire-bottomed to limit coprophagy) and maintained in temperature-controlled rooms (22°) with the dark period from 20.00 to 08.00 hours. Daily food consumption and body weight were recorded every 3 d during the first week, then daily during the period of digestive balance determination. Faeces were collected and weighed over four consecutive 24 h periods for studies on minerals excretion.

Sampling procedures

The rats were slaughtered just after the dark period (between 08.00 and 09.00 hours). After anaesthesia (40 mg sodium pentobarbital/kg), blood samples were taken from the caecal vein (0.5 ml, at a rate of 0.5 ml/min) and then the artery, as described previously (Demigné & Remesy, 1985). For blood flow measurement, bromosulphophthalein in saline (5 mmol/l) was infused into a small vein on the internal curvature of the caecum at a rate of 50 μ l/min: determination of the marker dilution in the vein draining the whole caecum (without collateral circulation to ileum or colon) was used to calculate the caecal blood flow. After blood sampling, the fat present on the caecum was removed and the caecum, complete with contents, was removed and weighed (total caecal weight). Duplicate samples

Table 1. *Composition (g/kg) of the experimental diets*

Ingredients	Dietary starch...	Digestible		Resistant	
	Ca level (g/kg)...	2.5	7.5	2.5	7.5
Wheat starch		742	716	392	376
Crude potato starch		0	0	350	350
Casein		150	150	150	150
Maize oil		50	50	50	50
Mineral mixture*†		40	40	40	40
Vitamin mixture†‡		10	10	10	10
CaCO ₃		1.25	3.75	1.25	3.75
CaHPO ₄		6.8	20.4	6.8	20.4

* Supplied (mg/kg diet): KCl 6 g, NaCl 6 g, MgCl₂·6H₂O 6.8 g, Fe₂O₃ 2.5, MnSO₄ 125, CuSO₄·7H₂O 0.2, ZnSO₄·7H₂O 100, KI 0.4. In both groups, Ca was supplied as CaCO₃ and CaHPO₄ to have a Ca:P_i of 1.25.

† Vitamins supplied (mg/kg diet): thiamin 20, riboflavin 15, pyridoxine 10, nicotinamide 100, calcium pantothenate 70, folic acid 5, biotin 0.3, cyanocobalamin 0.05, retinyl palmitate 1.5, DL- α -tocopheryl acetate 125, cholecalciferol 0.15, menadione 1.5, ascorbic acid 50, *myo*-inositol 100, choline 1.36 g.

‡ Both vitamin and mineral mixes were purchased from UAR (Villemoisson, Epinay-sur-Orge, France).

of caecal contents were placed in 2 ml microfuge tubes and stored immediately at -20° , then the caecal wall was flushed clean with ice-cold saline, blotted on filter paper and weighed (caecal wall weight). Supernatant fractions of the caecal contents were obtained by centrifuging the microfuge tubes at 20000 g for 10 min at 4° .

Analytical procedures

SCFA were determined by gas-liquid chromatography of portions of supernatant fractions of caecal contents (Demigné *et al.* 1980). Lactic acid concentrations (L- or D-forms) were measured using the reaction catalysed by the L(+) or D(-) dehydrogenases (*EC* 1.1.1.27 and *EC* 1.1.1.28 respectively) under conditions described by Gutmann & Wahlefeld (1974), after treatment of caecal supernatant fractions with 10 vol. 0.4 M-HClO₄. Mg, Ca and inorganic phosphate (P_i) were determined on the caecal supernatant fractions (soluble) and after mineralization (0.8 M-HCl, 12 h at 800°) of the untreated caecal samples (total). For Ca and Mg analysis the resulting residue was extracted with 5 M-HCl and made up to an appropriate volume with LaCl₃ solution (1 g/l). Mg and Ca were measured by atomic absorption spectrophotometry (Perkin-Elmer 420, Norwalk, CT) in an acetylene-air flame at a wavelength of 285 and 422 nm respectively. P_i was measured by a colorimetric method at 690 nm using a commercial kit (Biotrol, Paris, France). The previously described methods were checked against reference material from National Institute of Standards and Technology (Standard Reference Material 1548); the coefficient of variation was estimated for Ca, Mg and P_i respectively at 1.8, 1.6 and 2.0% for a diet matrix and at 2.2, 1.9 and 2.5% for a faeces matrix.

Calculations and statistical analyses

The entire caecal contents were determined as caecal concentration ($\mu\text{mol/ml}$) \times caecal water (ml), and the rate of caecal absorption (at the time of the measurement) as caecal vein - artery difference ($\mu\text{mol/ml}$ plasma) \times caecal plasma flow (ml/min). For the determination of digestive balance, food and faecal samples from each rat, collected over 4 d, were homogenized before mineral analysis. Intake of Ca via the drinking water was considered to be negligible (< 0.5 mg/l) compared with that from the diet and was not considered.

Values are given as means with their standard errors and, where appropriate, significance

of differences between mean values was determined by two-way ANOVA. Statistical analyses of the data were carried out according to the methods described by Snedecor & Cochran (1989). When data for the treatment groups did not meet the assumption of equal variance, observations were transformed logarithmically and these transformed values were used for the subsequent statistical examinations. Values of $P < 0.05$ were considered significant.

RESULTS

Changes in food intake, body weight and caecal digestion

Diets containing RS were well tolerated by the rats, provided that the level of 350 g raw potato starch/kg in the food was progressively reached, within 3–4 d, at the beginning of the 15 d adaptation period. As shown in Table 2, the daily food intake was not significantly affected by the diet, but the weight gain was slightly lower in rats adapted to the RS diets than in those fed on the DS diets. In contrast, dietary Ca intake had practically no effect on food intake or the growth of the rats.

The presence of 350 g raw potato starch/kg in the diet resulted in an enlargement of the caecum, corresponding to a 3–4-fold increase in the caecal contents together with hypertrophy of the caecal wall (Table 2). In rats fed on the RS diets the caecum was slightly heavier in animals fed at the low-Ca intake, compared with those fed at the high-Ca intake. Caecal blood flow was markedly higher in rats fed on RS diets (in the range of 3.2–3.5 ml/min *v.* about 0.9 ml/min with the DS diets); the dietary Ca level had no noticeable effect on caecal blood flow. The dry matter level in the caecal contents was significantly higher in rats fed on the RS diets. Caecal pH was close to neutrality in rats fed on the DS diets, and it was not influenced by the dietary Ca level. In contrast, dietary Ca intake influenced the acidification of caecal contents in rats fed on RS diets: the pH fell to 5 in rats adapted to 2.5 g Ca/kg diet compared with 5.7 with the high dietary Ca intake.

Table 3 shows that some SCFA were produced from endogenous substrates in rats fed on DS diets, at a pH close to neutrality. In the case of acidic fermentation (rats fed on RS diets) the dietary Ca intake markedly influenced the quantities of organic acids present in the caecum. In rats adapted to the 7.5 g Ca/kg diet there was a high concentration of SCFA in the caecum (approximately 160 mmol/l) together with lactic acid (approximately 80 mmol/l). With the low-Ca diet the very acidic fermentation was characterized by a lower concentration of SCFA (approximately 100 mmol/l) but lactic acid concentration was still in the range of 80 mmol/l. Due to the differences in caecal weight between the two dietary Ca levels, the entire caecal content of SCFA was higher with the 7.5 g Ca/kg diet and that of lactic acid was higher with the 2.5 g Ca/kg diet. Nevertheless, the caecal content of monocarboxylic acids (SCFA+lactic acid) was not markedly different (in the range 1300–1400 μ mol) between the two dietary Ca levels.

In rats fed on the DS diets the molar ratio of caecal SCFA (acetate:propionate:butyrate) was about 62:25:13, without any effect of dietary Ca. In rats fed on the RS diet with 2.5 g Ca/kg, fermentation was characterized by a high proportion of acetic acid and low proportion of propionic acid. In rats fed on the RS diet with 7.5 g Ca/kg, along with a high SCFA concentration there was a different SCFA molar ratio (50:27:23), corresponding to higher concentrations of propionic and butyric acid (about 40 mmol/l). The high concentrations of lactic acid present in rats fed on RS diets were essentially in the form of L-lactic (L:D approximately 3) and this was not influenced by the dietary Ca intake.

Changes in caecal availability of calcium and inorganic phosphate

Dietary Ca influenced greatly the accumulation of Ca in the large intestine since this mineral, when present in excess, is essentially eliminated in the faeces. Rats fed on the DS diets had the same concentration of soluble Ca in caecal contents (about 12 mmol/l)

Table 2. *Effects of dietary resistant starch (RS) and of the calcium level on food intake, daily weight gain and variables of caecal development of rats*

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

Diets§	Daily food intake (g/d)		Daily wt gain (g/d)		Caecum wt (g)		Caecal wall wt (g)		Dry matter (g/kg)		Caecal pH		Caecal blood flow (ml/min)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
DS-Ca (g/kg): 2.5	24.6	0.8	5.8	0.6	2.97	0.25	0.51	0.04	160	9	7.24	0.06	0.90	0.10
	24.9	0.9	5.8	0.2	3.08	0.18	0.52	0.03	205†	8	7.41	0.06	0.94	0.10
RS-Ca (g/kg): 2.5	23.9	0.9	5.2	0.2	12.59‡	1.20	2.38‡	0.14	275‡	16	5.05‡	0.04	3.49‡	0.16
	24.6	1.0	5.3	0.1	10.23‡	0.72	1.97††	0.12	307‡	16	5.72††	0.07	3.21‡	0.14
Significance of effects by ANOVA														
Starch	NS		NS		***		***		***		***		***	
Ca	NS		NS		NS		*		*		***		NS	
Starch × Ca	NS		NS		NS		*		NS		***		NS	

DS, digestible starch.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Mean values for groups fed on 2.5 g Ca/kg were significantly different from those of groups fed on 7.5 g Ca/kg: † $P < 0.05$.

‡ Mean values for groups fed on DS diets were significantly different from those fed on RS diets: ‡ $P < 0.05$.

§ For details of composition, see Table 1 and p. 302.

Table 3. *Effects of dietary resistant starch (RS) and of the calcium level on the caecal fermentation of rats*
(Mean values with their standard errors for ten rats per dietary group)

Diets§	Caecal concentration (mmol/l)												Total caecal pool (µmol/caecum)							
	Acetate		Propionate		Butyrate		Total SCFA		L-Lactate		D-Lactate		LD-Lactate		SCFA		Lactate			
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM		
DS-Ca (g/kg): 2.5	63.6	3.5	25.5	1.7	14.9	1.3	109.9	6.7	—	—	—	—	—	—	—	—	—	—	—	
7.5	63.2	1.1	24.3	1.1	11.9†	0.5	99.4	1.7	—	—	—	—	—	—	—	—	—	—	—	
RS-Ca (g/kg): 2.5	61.3	2.9	17.8‡	1.0	19.0‡	1.2	98.1	3.3	60.6	3.8	19.9	2.1	80.5	5.1	733‡	16	601	14	14	
7.5	80.0††	5.2	44.1††	2.4	37.5††	2.7	161.6††	8.9	58.5	3.8	19.5	2.2	78.0	4.7	936††	18	452†	11	11	
Significance of effects by ANOVA																				
Starch	*		***		***		***												***	
Ca	**		***		***		***												***	
Starch × Ca	**		***		***		***												***	

DS, digestible starch; SCFA, short-chain fatty acids.
 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
 Mean values for groups fed on 2.5 g Ca/kg were significantly different from those of groups fed on 7.5 g Ca/kg; † $P < 0.05$.
 Mean values for groups fed on DS diets were significantly different from those fed on RS diets; ‡ $P < 0.05$.
 § For details of composition, see Table 1 and p. 302.

Table 4. Effect of dietary resistant starch (RS) and of the calcium level on the caecal accumulation and the solubility of Ca and inorganic phosphate (P_i) in rats
(Mean values with their standard errors for ten rats per dietary group)

Diets§	Ca						P _i														
	Total (mmol/l)			Soluble (mmol/l)			Solubility (% of total)			Total caecal pool (µmol/caecum)			Soluble (mmol/l)			Solubility (% of total)			Total caecal pool (µmol/caecum)		
	Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM	
DS-Ca (g/kg): 2.5	157	4	12.6	1.0	8	374	14	105	4	4.2	0.9	4	250	10							
	640†	11	11.9	0.8	2	1574†	49	416†	14	3.2	0.3	1	1023†	39							
RS-Ca (g/kg): 2.5	32‡	3	15.7	1.6	49	330	16	46‡	2	20.8‡	1.6	45	474‡	17							
	245†‡	8	40.2†‡	2.4	16	2048†‡	85	184†‡	8	27.3†‡	2.8	15	1538†‡	79							
Significance of effects by ANOVA	***		***		—	*		***		***		***		***							
Starch	***		***		—	***		***		NS		—		***							
Ca	***		***		—	*		***		NS		—		***							
Starch x Ca	***		***		—	*		NS		NS		—		**							

DS, digestible starch.
 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
 Mean values for groups fed on 2.5 g Ca/kg were significantly different from those of groups fed on 7.5 g Ca/kg; † $P < 0.05$.
 Mean values for groups fed on DS diets were significantly different from those fed on RS diets; ‡ $P < 0.05$.
 § For details of composition, see Table 1 and p. 302.

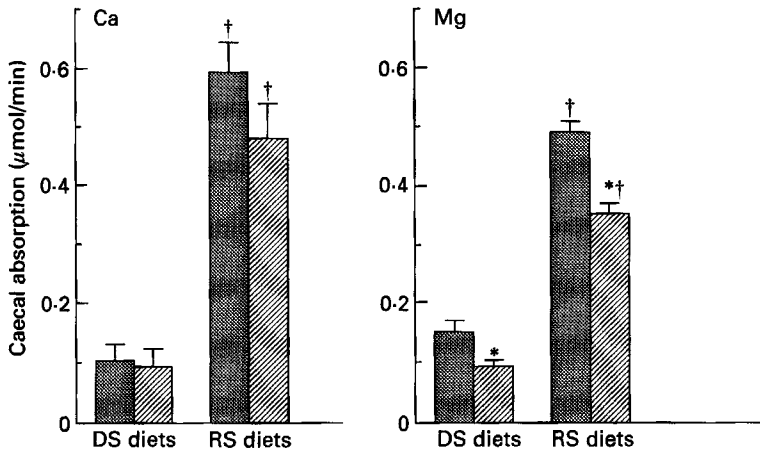


Fig. 1. Changes in calcium or magnesium absorption from the caecum of rats adapted to digestible-starch (DS) or resistant-starch (RS) diets, and to a 2.5 (▨) or a 7.5 (▩) g Ca/kg level. For details of diets and procedures, see pp. 302–303 and Table 1. Values are means with their standard errors, represented by vertical bars, for ten rats per group. Mean values for groups of rats fed on 7.5 g Ca/kg were significantly different from those for groups of rats fed on 2.5 g Ca/kg: * $P < 0.05$. Mean values for groups of rats fed on the RS diet were significantly different from those for groups of rats fed on the DS diet: † $P < 0.05$.

whatever the dietary Ca level (Table 4). At neutral pH, Ca was mainly insoluble (in the form of Ca- P_i complex) and the P_i concentration (and solubility) varied in parallel to that of Ca, with a relatively constant Ca: P_i value of about 1.5. The caecal accumulation of Ca- P_i was considerable in rats fed on the DS-high-Ca diet (1574 and 1023 μmol for Ca and P_i respectively). In rats fed on the RS diets the total Ca was diluted in a large volume; at 2.5 g Ca/kg a particularly low Ca concentration was found in the caecum (32 mmol/l). In rats fed on the low Ca level the caecal concentration of soluble Ca (16 mmol/l) was not noticeably affected by feeding RS. However, the percentage of soluble Ca was dramatically increased (from 8 in rats fed on DS diets to 49 in rats fed on RS diets). The entire caecal content of Ca was significantly increased by RS only in rats adapted to the 7.5 g Ca/kg level. Ca solubility was also enhanced in rats fed on the RS-high-Ca diet; the soluble Ca reached 40 mmol/l compared with 12 mmol/l in rats fed on DS diets. Dietary RS also affected the quantities and the solubility of P_i in caecal contents. As for Ca, a decrease in P_i concentration was observed in rats fed on RS diets, in parallel with a 3–4-fold enlargement of the caecum; the accumulation of P_i was markedly higher with the high Ca level. Changes in soluble P_i concentrations were relatively parallel with those of Ca, the percentage of soluble P_i being higher in rats fed on the RS diets than in control (DS-fed) rats. The elevation of the P_i solubility by the RS diet was particularly significant with the low Ca level (from 4 to 45%).

Digestive absorption of calcium and inorganic phosphate

In the rat there is an efficient absorption of Ca by the caecum, which can be quantified by measurement of arterio-venous differences (Fig. 1); in contrast, P_i was not absorbed in significant amounts. In rats fed on DS diets Ca absorption by the caecum was low and was not responsive to changes in the level of dietary Ca. Rats fed on RS diets had a 5–6-fold higher caecal Ca absorption than rats fed on DS diets; however, there was no significant difference between the two Ca levels, despite a marked difference in soluble Ca in the caeca of rats adapted to RS. It must be noted that this rise in the caecal absorption of Ca was a reflection of both accelerated blood flow (+250–300%) and greater arterio-venous differences (+30–50%). Table 5 shows that, in control rats, the difference (Ca intake–Ca

Table 5. *Effects of dietary resistant starch (RS) and of the calcium level on the daily intake and faecal excretion of calcium and inorganic phosphate (P_i) in rats*

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

Diets§	Ca										P _i				
	Daily intake (DI) (mg/d)		Faecal excretion (FE) (mg/d)		DI-FE difference				DI (mg/d)		FE (mg/d)		DI-FE difference		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
					mg/d		% of intake						mg/d		% of intake
DS-Ca (g/kg): 2.5	74.8	3.5	36.1	2.0	38.7	1.9	52	56.7	3.2	23.3	1.1	33.4	2.0	59	
7.5	225.3†	5.4	183.8†	2.8	41.5	1.5	18	132.7†	4.0	110.5†	3.6	22.2†	0.9	17	
RS-Ca (g/kg): 2.5	72.5	3.3	25.0‡	1.9	47.0‡	2.4	65	65.0	3.5	34.6‡	1.5	30.4	1.8	47	
7.5	221.6†	6.1	175.9†	3.1	45.7‡	1.3	21	143.4†	4.6	124.5††	4.2	18.9†	0.7	13	
Significance of effects by ANOVA															
Starch	NS		*		**		—	*		***		*		*	
Ca	***		***		NS		—	***		***		***		***	
Starch × Ca	NS		**		NS		—	NS		**		NS		NS	

DS, digestible starch.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Mean values for groups fed on 2.5 g Ca/kg were significantly different from those of groups fed on 7.5 g Ca/kg: † $P < 0.05$.

Mean values for groups fed on DS diets were significantly different from those fed on RS diets: ‡ $P < 0.05$.

§ For details of composition, see Table 1 and p. 302.

Table 6. *Effects of dietary resistant starch (RS) and of the calcium level on magnesium digestive balance in rats*

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

Diets§	Daily intake (DI) (mg/d)		Faecal excretion (FE) (mg/d)		DI-FE difference			Plasma concentration (mmol/l)	
	Mean	SEM	Mean	SEM	mg/d		% of intake	Mean	SEM
					Mean	SEM			
DS-Ca (g/kg): 2.5	20.1	0.9	12.0	0.7	8.1	0.4	40	0.95	0.04
7.5	21.1	1.1	17.0†	0.8	4.1†	0.3	19	0.81†	0.03
RS-Ca (g/kg): 2.5	20.2	1.0	8.1‡	0.6	12.1‡	0.9	60	0.92	0.03
7.5	21.3	1.3	14.1†‡	1.0	7.2†‡	0.7	34	0.86	0.04
Significance of effects by ANOVA									
Starch	NS		***		***		—	NS	
Ca	NS		***		***		—	**	
Starch × Ca	NS		NS		NS		—	NS	

DS, digestible starch.

** $P < 0.01$, *** $P < 0.001$.† Mean values for groups fed on 2.5 g Ca/kg were significantly different from those of groups fed on 7.5 g Ca/kg; $P < 0.05$.‡ Mean values for groups fed on DS diets were significantly different from those fed on RS diets; $P < 0.05$.

§ For details of composition, see Table 1 and p. 302.

faecal excretion) was not affected by increasing the dietary Ca intake. Furthermore, in rats fed on the high-Ca diets the presence of RS in the diet failed to alter this difference. However, rats fed on a RS diet with a low Ca level showed a higher (Ca intake—Ca fecal excretion) difference, compared with rats fed on the low-Ca—DS diet. Despite changes in the availability of dietary Ca or of complex carbohydrate, plasma Ca concentration was unchanged in the experimental groups (values not presented). A high dietary intake of Ca depressed significantly the P_i difference (intake—faecal excretion) difference, probably because Ca tends to attract P_i into the large intestine. RS failed to counteract significantly the negative effect of Ca on P_i .

Change in caecal magnesium availability and in magnesium absorption

In rats adapted to DS diets, the dietary Ca level had no effect on the amount of Mg present in caecal contents (range 230–255 μmol); nevertheless, Mg solubility was lower in rats fed on diets containing a high Ca level, in parallel with Ca- P_i accumulation. In rats fed on RS diets a higher volume of caecal contents reduced Mg concentration, from about 100 mmol/l in rats fed on the DS diets to about 25 mmol/l in those fed on the RS diets (values not presented). The caecum appeared to be a site of Mg absorption, even in rats fed on the DS diets (Fig. 1). The enlargement of the caecum in rats fed on RS diets corresponded to a 3–4-fold higher caecal Mg absorption compared with control (DS) rats. A high Ca intake resulted in a lower Mg absorption by the caecum, consistent with the fall in soluble Mg described previously.

As with caecal absorption, dietary Ca exerted a negative effect on Mg digestibility, which was 40 or 19% respectively in rats adapted to DS diets containing 2.5 or 7.5 g Ca/kg, (Table 6). Compared with the DS diet the RS diet markedly improved Mg digestive balance; Mg digestibility was higher with the low dietary Ca level. In rats fed on RS diets

the dietary Ca level had no noticeable effect on plasma Mg; however, magnesaemia was significantly depressed by the high Ca level when rats were adapted to DS.

DISCUSSION

Compared with some soluble fibres RS is devoid of uronic acids which can chelate divalent cations. Thus, the effects of RS on mineral digestibility seems essentially a consequence of colonic fermentation. The present work also indicates that the bioavailability of major minerals, especially Ca and P_i , plays an important role in the stability of symbiotic fermentation (SCFA-rich type) and the control of lumen pH in the large intestine. In contrast, fermentation may result in a higher absorption of divalent cations.

Enlargement of the caecum in rats consuming unavailable carbohydrates (fermentable fibres or RS) has been reported consistently (Würsch, 1989; Goodlad & Mathers, 1990; Levrat *et al.* 1991*b*). The effect of complex carbohydrates on caecal hypertrophy tends to be proportional to their fermentability rather than merely to their accumulation in the caecum; accordingly, poorly-fermentable fibres (e.g. oat hull fibre) have little influence on the caecum size (Rémésy *et al.* 1992). In the present study the caecum of rats fed on RS diets (digesta contents + caecal wall) was markedly enlarged (3–4-fold), which results in a dilution of minerals coming from the small intestine and in a greater surface area for absorption. Under such conditions it has been reported that the height of crypt columns and the number of cells per crypt is markedly enhanced (Rémésy *et al.* 1993). SCFA probably represent important stimuli for the colonic cell proliferation frequently observed after feeding fibre (Lupton & Kurtz, 1993); it is conceivable that this trophic effect might result in a more effective absorption of Ca.

There is a permanent influx of minerals into the large intestine from the ileum, and the dietary level of the minerals certainly influences their bioavailability in the caecum. This appears particularly relevant for Ca; with a high dietary level of Ca large amounts of this cation tend to accumulate in the large intestine, and the major part of Ca is probably present as an insoluble Ca- P_i complex at physiological pH. The P_i concentration appears essentially governed by that of Ca; the Ca: P_i ratio was generally > 1 , especially in rats fed on the high-Ca-fibre-free (DS) diet (Ca: P_i ratio: 1.54). The presence in the caecum of a pH-buffering system involving the Ca- P_i pool has been described in rats fed on inulin (Rémésy *et al.* 1993). Such a pool of insoluble Ca (devoid of osmotic effect) may be effective in counteracting caecal acidification when there is very active fermentation. Schulz *et al.* (1993) have reported also that RS lowers the pH and raises the Ca concentration in caecal water. In rats fed on DS diet the buffering capacity of caecal contents was very high, even with the low-Ca diet (total Ca 157 mmol/l), because electrolytes from the ileal effluent were concentrated in a small volume. In contrast, in rats adapted to the low-Ca-RS diet, effective absorption of Ca in the upper digestive tract, together with a dilution of unabsorbed Ca in a large caecal volume, led to lower caecal Ca and P_i concentrations (30–40 mmol/l). Generally, when a variety of dietary fibre is fermented in the caecum the main endproducts of bacterial fermentations are SCFA, with a marginal production of lactic acid (Rémésy *et al.* 1992, 1993). The presence of large quantities of fermentable substrates in the caecum of rats fed on raw potato starch offered conditions favourable to the production of both SCFA and lactic acid isomers. It is generally accepted that a very acidic pH (about 5) inhibits micro-organisms which metabolize lactic acid (Cummings, 1981); yet, a noticeable production of lactic acid was also observed at pH 5.7 (7.5 g Ca/kg level). A direct influence of minerals on caecal fermentation is still uncertain; it is noteworthy that propionic and butyric acid concentrations were significantly depressed in the caecum of rats fed on the low-Ca diet, but this could result from excessive acidification.

Ca absorption in the small intestine is strictly regulated (Bronner *et al.* 1986); thus, the

quantities of Ca reaching the large intestine are not directly proportional to the dietary Ca intake. With the 2.5 g Ca/kg level, the disappearance of Ca in the small intestine was high and RS seems to have little effect on this process. In contrast, there was a net increase in Ca present in the caecum of rats fed on the RS diet containing 7.5 g Ca/kg. Hypertrophy of the caecum at acidic pH with RS diets strongly stimulates Ca absorption in distal absorptive sites. A high rate of Ca absorption in the large intestine could trigger a feedback mechanism involving an inhibition of duodenal absorption, since there is a control of the digestive balance of Ca by endocrine factors (Nellans & Goldsmith, 1981; Bronner *et al.* 1986). The rat caecum presents the highest density of Ca transport sites (responsive to vitamin D metabolites); however, Ca absorption is restricted when Ca is in an unabsorbable form (Nellans & Goldsmith, 1981; Amman *et al.* 1986). Thus, fermentable carbohydrates could favour Ca absorption in the distal part of the digestive tract in several ways: hypertrophy of the caecal wall and greater surface area, increase in soluble Ca, and accelerated blood flow.

Although the affinity of the Ca transport system seems high (K_t value at $\frac{1}{2}V_{max}$ being approximately 1 mM; Favus, 1985), the solubilization of Ca by organic acids probably plays an essential role in the enhanced contribution of the caecum to overall digestive absorption (caecal soluble Ca 24 μmol in both DS-diet groups, compared with 120 and 230 μmol in rats fed on RS diets containing 2.5 or 7.5 g Ca/kg diets respectively). Moreover, an increase in total transport sites due to caecal hypertrophy could elevate the rate of absorption of solubilized Ca. It is possible also that SCFA directly influence Ca absorption by modifying various electrolyte exchanges (Ca-H) and Trinidad *et al.* (1993) have proposed that Ca could pass through the cell membrane more readily in the form of a less-charged complex (CaAc)⁺. Lutz & Scharrer (1991) have also reported a stimulatory effect of SCFA on Ca absorption in the rat large intestine. In fact, there are probably several factors which affect Ca absorption (caecal pH, SCFA concentration, Ca itself) and the question arises whether SCFA have a direct effect on the lumen or affect passive Ca absorption due to the hypertrophy of the caecum.

Ca availability in the large intestine has been the subject of various investigations since it may exert a protective effect on the colon epithelium (Wargovich *et al.* 1983) and inhibit the cytotoxicity of potential carcinogens, such as bile acids or fatty acids (Lapre *et al.* 1991; Gorvers & Van der Meer, 1992). It must be noted that in rats adapted to the low-Ca-RS diet butyric acid and propionic acid concentrations were depressed, which may be physiologically relevant since these acids (especially butyric acid) are considered as inhibitors of cell proliferation as well as differentiation inducers in several types of cancer cells (Kruh, 1982; Gamet *et al.* 1992).

Compared with Ca, the importance of the distal part of the digestive tract for Mg absorption is well documented (Hardwick *et al.* 1990; Karbach & Rummel, 1990) and it has been shown previously that various types of RS stimulate Mg absorption (Rayssiguier & Révész, 1977; Schulz *et al.* 1993). As with Ca, fermentable carbohydrates may raise the pool of soluble Mg in the large intestine by acidifying digesta contents (Mg solubility being generally higher than that of Ca). It is noteworthy that Mg solubility tends to fall when large amounts of insoluble Ca-P_i complex are present in the caecum. The striking effect of RS diets on caecal absorption of Mg (4-fold increase) probably resulted from (1) caecal hypertrophy, (2) Mg solubilization and (3) possibly, a specific effect of SCFA (Scharrer & Lutz, 1992). Indeed, SCFA are predominantly absorbed in an undissociated form in the large intestine, although they mainly occur as anions in the lumen (Rechkemmer *et al.* 1988). Protons needed for SCFA absorption may be delivered by various ion exchangers (including Mg-H); in return SCFA absorption at acidic pH would supply more protons to the exchangers, resulting in a higher transport rate (Lutz *et al.* 1991). Feeding RS had a

greater effect on the apparent digestive balance of Mg than on that of Ca. The possibility of a negative feedback in response to a highly effective absorption in the large intestine seems less likely for Mg than for Ca. This is in accordance with the fact that Mg absorption is more efficient in the ileum and the large intestine (Hardwick *et al.* 1990). A high dietary Ca supply consistently led to an inhibition of Mg absorption in the caecum and a reduction in Mg digestibility. In contrast to the divalent cations, it was not possible to detect any significant absorption of P_i in the large intestine (by arterio-venous difference). Since high Ca concentrations promote precipitation of P_i and inhibit its intestinal absorption, this P_i transfer into the large intestine depressed the digestive balance of P_i . This balance was not affected by RS feeding despite increased caecal content of P_i .

In conclusion, the enhancement of Ca absorption in the large intestine by increasing colonic fermentation may be of particular interest when the overall process of digestive absorption is inefficient, such as in elderly subjects (Andon *et al.* 1993). In humans, a role for the colon is supported by the observation that the large intestine is able to maintain a near-normal rate of Ca absorption in cases of small intestinal resection (Hylander *et al.* 1980). In humans, RS may also lower the colon lumen pH, even if the daily intake is much lower than that in the present work (Englyst *et al.* 1992) and the fermentation rate of raw potato starch is low (Olesen *et al.* 1994). The Ca content of the human diet is usually about 1 g/kg, i.e. much less than that in the present study; nevertheless, such a dietary supply leads to the accumulation of high Ca- P_i concentrations in human faeces (Lapr e *et al.* 1991) and it seems likely that Ca solubility in the large intestine is also influenced by polysaccharide fermentation. Simultaneous supplementation of the diet with Ca and fermentable carbohydrate (RS and/or plant foods rich in soluble fibre) seems advantageous to promote fermentation, to counteract the negative effect of dietary Ca on Mg digestibility and to stimulate Ca absorption in the large intestine. Finally, microbial fermentation appears to have a major influence on the efficiency of Ca absorption in the large intestine.

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