Increases in calcium absorption with ingestion of soluble dietary fibre, guar-gum hydrolysate, depend on the caecum in partially nephrectomized and normal rats

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Effects of feeding soluble dietary fibre on apparent Ca absorption and the contribution of the caecum to Ca absorption were examined in five-sixths nephrectomized (NPX) and normal rats with or without caecectomy in four experiments. It is known that Ca absorption is lowered by renal failure. In the first experiment the amounts of femur Ca increased linearly with increasing dietary Ca up to 3·0 g Ca/kg diet in intact rats. Partial nephrectomy decreased apparent Ca absorption in rats fed on diets containing 3·0 and 4·5 g Ca/kg diet. In the NPX groups, Ca absorption in rats fed on the diet containing guar-gum hydrolysate (GGH; 50 g/kg diet; 3·0 g Ca/kg diet) was significantly higher than that in rats fed on a fibre-free diet, and the increase in Ca absorption with GGH feeding was completely abolished by caecectomy. Also, ingestion of GGH increased Ca absorption in normal rats, but not in normal, caecectomized rats. Mg absorption was also increased with GGH feeding and was decreased with caecaectomy in NPX and normal rats. In experiments which used caecectomized rats, coprophagy was prevented with an anal cup to avoid re-ingestion of faecal Ca. We conclude that ingestion of the soluble dietary fibre, GGH, increased apparent Ca absorption in NPX and non-NPX rats, and the caecum was responsible for these increases in Ca absorption.

Calcium: Dietary fibre: Caecum

Low intake and low absorption rate of dietary Ca are involved in osteoporosis. In human subjects the rate of Ca absorption from diets is relatively low and absorption is impaired by ageing, menopause and renal failure (Kaye & Silverman, 1965; Coburn *et al.* 1973). Other dietary components also influence Ca absorption. The reported effects of dietary fibre on Ca absorption are controversial and include: a negative effect on Ca transport (Oku *et al.* 1982) or apparent Ca absorption (Donangelo & Eggum, 1986), no effect of fibre on Ca status (van der Aar *et al.* 1983), and a stimulatory effect on Ca absorption in the caecum of rats fed on a fibre-containing diet (Demigné & Rémésy, 1985; Demigné *et al.* 1989).

There has been considerable debate over the major site of Ca absorption in the gastrointestinal tract. Different results have been reported under various experimental conditions: Ca absorption predominates in the duodenum (Annaka *et al.* 1989), the jejunum (Hurwitz & Bar, 1966), the ileum (Marcus & Lengemann, 1962) and the proximal colon (Karbach & Feldmeier, 1993).

The purposes of the present study were to examine the effects of a soluble dietary fibre, guar-gum hydrolysate (GGH), on lowered Ca absorption in rats with renal failure and to determine the contribution of the large intestine to Ca absorption by comparing caecectomized (CCX) rats with normal rats. The study comprised four separate experiments: effects of Ca levels in diets, effects of renal failure, effects of feeding fibre in rats with renal failure, and the corresponding effects in normal rats. We used five-sixths nephrectomized (NPX) rats as a model of renal failure to quantitatively impair renal functions, and used GGH as a soluble low-viscosity fibre source to minimize the physical effects of fibre in the upper alimentary tract. Also, coprophagy was prevented to exclude the possibility that re-ingested Ca in faeces was absorbed from the small intestine in the experiments in which the effects of caecectomy and feeding dietary fibre were examined.

EXPERIMENTAL METHODS

Animals and diets

Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan), weighing about 100 g, were given free access to deionized water and a semi-purified stock diet (Table 1) for 5 d to acclimatize animals, and were divided into several groups in a randomized block design, based on body weight, in four separate experiments.

In the studies using CCX rats, coprophagy was prevented by the use of an anal cup as described previously (Ohta *et al.* 1996). Rats used in all experiments were housed individually in stainless-steel cages with mesh bottoms. The cages were placed in a room with controlled temperature (22–24°), relative humidity (40–60 %) and lighting (light 08.00–20.00 hours).

The five-sixths nephrectomy (Morrison, 1962) consisted of two operations. At the first stage of the procedure, renal blood vessels were clamped and two-thirds of the left kidney was removed, and bleeding was stopped by a surgical adhesive (α-cyanoacrylate). The clamp was released within 2 min. After 1 week of the first stage the right kidney was excised via a small incision (10–15 mm) after ligation of the renal blood vessels and the ureter. Body temperature was maintained at 37–40° throughout the operations by a warmed surgical plate. Survival rates of rats with the nephrectomy were 67 % in Expt 3 and 78 % in Expt 4.

GGH (Sun fibre; Taiyo Kagaku Co. Ltd, Yokkaichi, Japan) is a partial hydrolysate of guar-gum produced by β-1,4-mannanase (*EC* 3.3.1.78) and has an average molecular weight of 15000. The fibre source (50 g/kg diet) was added to a test diet containing 3·0 g Ca/kg diet at the expense of whole diet. Rats had free access to the test diets and deionized water during the experimental period. Body weight and feed intake were measured every day. Spilled diet was carefully collected and weighed. Feed intakes for experimental periods were corrected for the feed spilled.

Faeces were collected during the last 3 d in each experiment to evaluate Ca and Mg excretion and apparent absorption of Ca and Mg. In Expts 1 and 2 the faeces were sampled for 3 d using stainless-steel wire mesh placed under the bottom of the cages, and in Expts 3 and 4, faeces were collected for 3 d from an anal cup. The faeces collected were freeze-dried.

At the end of each experiment, rats were killed under anaesthesia with Nembutal (pentobarbital sodium 50 g/l; Abbott, North Chicago, IL, USA). The right femur was removed and carefully cleaned of adherent tissue.

The study was approved by the Hokkaido University Animal Committee, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Table 1. *Composition (g/kg diet) of stock and test diets*

	Stock and test diets*
Casein†	150
L-Methionine	3·0
Maize oil‡	50
Mineral mixture§	40
Vitamin mixture‖	10
Granulated vitamin E¶	1·0
Choline bitartrate	4·0
Sucrose	To make 1 kg

* Ca concentration in a stock diet was 4·5 g/kg diet. The composition of test diets used in Expts 1–4 was the same as that of the stock diet except for the Ca concentration. Guar-gum hydrolysate (50 g/kg diet) was added to the test diet containing 3·0 g Ca/kg diet. Crystallized cellulose (Avicel; Asahi Chemical Industry Co. Ltd, Tokyo, Japan; 50 g/kg diet) was added to all test diets.

† Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).

‡ Retinyl palmitate (7·66 μmol/kg diet) and ergocalciferol (0·0504 μmol/kg diet) were added to the maize oil. Ergocalciferol was excluded from the test diets used in Expts 2–4.

§ The mineral was based on the AIN-76 mixture (Reeves, 1989) without Ca. It provided (mg/kg diet); P 2997, K 3746, Mg 375, Fe 100, I 0·32, Mn 10·0, Zn 34·7, Cu 6·00, Na 4279, Cl 6542, Se 1·05, Mo 1·00, Cr 0·50, B 0·50, V 0·25, Sn 2·00, As 1·00, Si 20·0, Ni 1·00, F 2·72, Co 0·20. Ca concentration varied: 0·1–4·5 g/kg diet in Expt 1, 3·0 and 4·5 g/kg diet in Expt 2, and 3·0 g/kg diet in Expts 3 and 4.

‖ The vitamin mixture was prepared in accordance with the AIN-76 mixture (American Institute of Nutrition, 1977) except that menadione and L-ascorbic acid were added at levels of 5·81 (American Institute of Nutrition, 1980) and 284 (Harper, 1959) μmol/kg diet respectively.

¶ Vitamin E granules (Juvela; Eisai Co., Tokyo, Japan) supplied 423 μmol DL-α-tocopheryl acetate/kg diet.

Experimental protocols

In Expt 1 (effects of Ca levels in diets), acclimatized rats, divided into six groups of six rats, were fed on diets containing six levels of Ca (0·1, 0·5, 1·0, 1·5, 3·0 and 4·5 g Ca/kg diet) for 3 weeks. To make graded levels of Ca in the diet, $CaCO_3$ was replaced with sucrose.

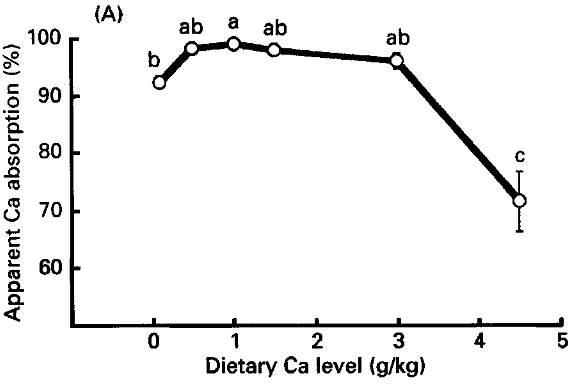
In Expt 2 (effects of nephrectomy), acclimatized rats were divided into two groups. One group of eighteen rats underwent nephrectomy as described previously. In twelve rats of another group, laparotomy was performed twice (sham operation; sham group). At 3 d after the 2nd operation each group was divided, on the basis of body weight, into two subgroups based on dietary Ca level (4·5 g/kg diet (Ca-sufficient) and 3·0 g/kg diet (Ca-insufficient); Table 1). The NPX and sham rats were fed on the test diets for 7 d.

In Expt 3 (effects of caecectomy in NPX rats), thirty-six acclimatized rats were divided into two groups, and these rats underwent nephrectomy. The caeca of one NPX group were resected during the first stage of nephrectomy, and the other NPX group was not caecectomized. The caecectomized (NPX-CCX) and non-caecectomized (NPX-sham) rats were divided into two subgroups 3 d after the last operation, and were fed on diets containing 3·0 g Ca/kg diet with or without GGH (50 g/kg diet) for 7 d.

In Expt 4, intact rats fed on a stock diet for 5 d were divided into two groups, one group of twelve rats underwent caecectomy (normal-CCX) and the other groups of twelve rats underwent laparotomy (normal-sham). Rats of each group were divided into two subgroups of six rats 10 d post-operatively, and were fed on diets containing 3·0 g Ca/kg diet with or without GGH (50 g/kg diet) for 7 d.

Analytical methods

Freeze-dried faeces were milled. Powdered faeces (about 70 mg) and freeze-dried whole femur were wet-ashed with nitric acid (10 mol/l) and perchloric acid (2·3 mol/l) mixture,



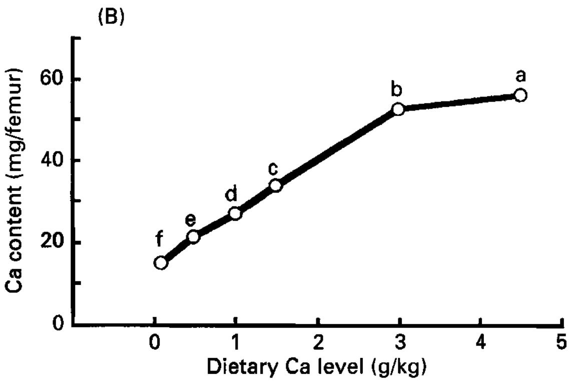


Fig. 1. Expt 1. Changes in apparent calcium absorption (A) and amount of Ca in the femur (B) of rats fed on diets containing graded levels of Ca. All values are means with their standard errors represented by vertical bars for six rats. The effect of dietary Ca level on Ca absorption and on the amount of Ca in the femur was significant (one-way ANOVA; $P < 0{\cdot}0001$). a, b, c, d, e, f Mean values with unlike superscript letters were significantly different between diet groups (Duncan's multiple-range test; $P < 0{\cdot}05$). For details of diets and experimental procedures, see Table 1 and pp. 774–776.

and the Ca and Mg concentrations of the ashed solution were measured by atomic absorption spectrophotometry (Spectr AA-20; Varian Techtron Pty Ltd, Mulgrave, Victoria, Australia) after appropriate dilution with deionized water.

Calculations and statistics

Each value was calculated as follows:

$$\text{apparent Ca (Mg) absorption (\%)} = 100 \times (\text{total Ca (Mg) intake} - \text{faecal Ca (Mg) excretion})/\text{total Ca (Mg) intake.}$$

The results of Expt 1 (effects of Ca levels) were analysed by one-way ANOVA. The effect of Ca level and nephrectomy (Expt 2), and the effects of dietary fibre and caecectomy (Expts 3 and 4) were analysed by two-way ANOVA. Duncan's multiple-range test or Student's t test was used to determine whether mean values were significantly different ($P < 0{\cdot}05$). These statistical analyses were done by GLM procedure of Statistical Analysis Systems (1989).

Table 2. *Expt 2. Changes in body-weight gain (g/d) and feed intake (g/d) during 7 d on the experimental diets containing 4·5 or 3·0 g calcium/kg diet for sham-operated (sham) and partially nephrectomized (NPX) rats**

(Mean values with their standard errors for six rats)

Ca level (mg/kg diet)	Treatment	Body-wt gain		Feed intake	
		Mean	SE	Mean	SE
4·5	Sham	7·70[a]	0·51	20·6[a]	0·67
4·6	NPX	5·09[b]	0·56	15·3[b]	1·06
3·0	Sham	7·71[a]	0·17	19·5[a]	0·56
3·0	NPX	5·26[b]	0·30	14·8[b]	1·28
Statistical significance (ANOVA) of the effect of:					
Ca level		$P = 0.6655$		$P = 0.3949$	
Treatment (T)		$P < 0.0001$		$P < 0.0001$	
Ca level × T		$P = 0.3953$		$P = 0.7794$	

[a, b] Mean values with unlike superscript letters were significantly different (Duncan's multiple-range test; $P < 0.05$).

* For details of diets and experimental procedures, see Table 1 and pp. 774–775.

Table 3. *Expts 3 and 4: Dry weight of faeces (g/3d) during a balance period for partially nephrectomized (NPX) (Expt 3) and normal rats (Expt 4) with or without caecectomy (CCX or sham respectively) fed on a fibre-free diet or a diet containing guar-gum hydrolysate (GGH)**

(Mean values with their standard errors for seven rats in Expt 3 and for six rats in Expt 4)

Treatment	GGH in diet	Dry weight of faeces				Feed intakes			
		NPX rats		Normal rats		NPX rats		Normal rats	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sham	–	2·46[b]	0·128	3·01[b]	0·148	37·6	3·20	46·4	1·69
Sham	+	2·32[b]	0·223	3·17[b]	0·134	36·6	3·00	44·4	2·72
CCX	–	2·58[b]	0·171	3·23[b]	0·242	37·0	4·15	45·9	2·10
CCX	+	4·25[a]	0·250	5·04[a]	0·136	43·0	1·50	47·0	1·65
Statistical significance (ANOVA) of effect of:									
Treatment (T)		$P < 0.0001$		$P < 0.0001$		$P = 0.9146$		$P = 0.6031$	
Diet		$P = 0.0008$		$P < 0.0001$		$P = 0.6871$		$P = 0.8311$	
T × diet		$P < 0.0001$		$P < 0.0001$		$P = 0.8835$		$P = 0.4748$	

[a, b] Mean values with unlike superscript letters were significantly different (Duncan's multiple-range test; $P < 0.05$).

* For details of diets and experimental procedures, see Table 1 and pp. 774–775.

RESULTS

In Expt 1, apparent Ca absorption was maintained at a high level with dietary Ca levels from 0·5 g/kg diet to 3·0 g/kg diet, and was markedly lowered with a sufficient level of dietary Ca (4·5 g/kg diet), as shown in Fig. 1. The amount of femur Ca increased linearly with increasing dietary Ca level from 0·1 g/kg diet to 3·0 g/kg diet. The rate of increase of femur Ca was slowed at more than 3·0 g Ca/kg diet. These results show that a level of

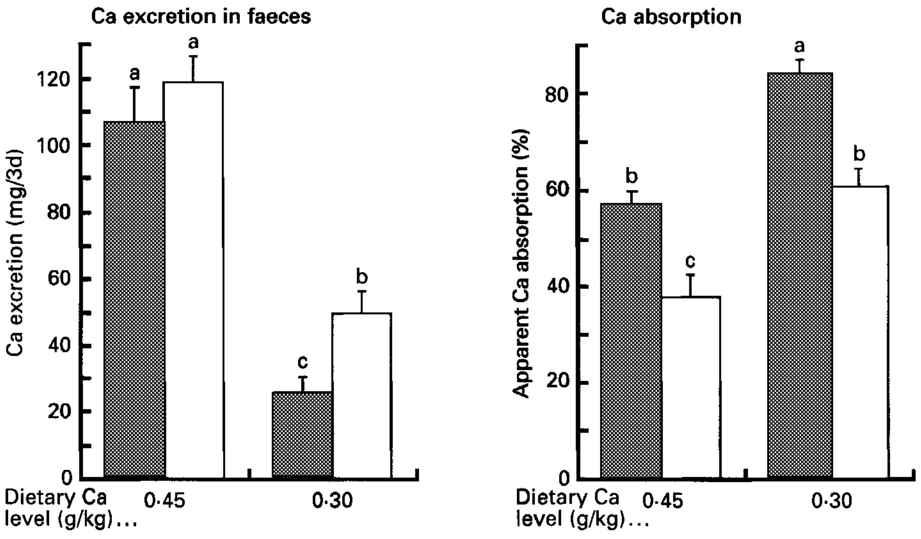


Fig. 2. Expt 2. Faecal excretion of calcium and apparent Ca absorption in normal (sham-operated; ▨) and partially nephrectomized (□) rats fed on diets containing 0·30 and 0·45 g Ca/kg. All values are means with their standard errors represented by vertical bars for six rats. The statistical significance of the effects of dietary Ca level (Ca), treatment (nephrectomy) and Ca × treatment respectively (two-way ANOVA) on faecal Ca excretion were $P < 0{\cdot}0001$, $P = 0{\cdot}0324$ and $P = 0{\cdot}4420$ and for apparent Ca absorption were $P < 0{\cdot}0001$, $P < 0{\cdot}0001$ and $P = 0{\cdot}5417$. a, b, c Mean values with unlike superscript letters were significantly different (Duncan's multiple-range test; $P < 0{\cdot}05$). For details of diets and experimental procedures, see Table 1 and pp. 774–776.

3·0 g Ca/kg diet is insufficient, but near a level of minimum requirements of $CaCO_3$ in rats, and up to that level bone Ca content reflects the amounts of Ca absorbed from the intestine.

In Expt 2, body-weight gain and feed intake during the 7 d experimental period were lower for NPX rats than for sham rats at both Ca levels (3·0 g and 4·5 g/kg diet; Table 2). Ca level did not affect either variable. In NPX and normal rats (Expts 3 and 4) in which coprophagy was prevented, body-weight gain and feed intake were not affected by caecectomy or by feeding GGH (two-way ANOVA; $P > 0{\cdot}3$). The average values for body-weight gain and feed intake were 3·34 and 13·6 g/d for NPX rats (*n* 28), and 7·28 and 15·3 g/d for normal rats (*n* 24) respectively.

Table 3 shows that ingestion of GGH increased the faecal DM of the caecectomized group, but not that of the sham group (non-CCX) in NPX and normal (non-NPX) rats. Feed intakes during a corresponding period (3 d) were not influenced by caecectomy or GGH feeding in NPX and normal rats.

Fig. 2 (results of Expt 2) shows that Ca absorption was higher in rats fed on the diet containing 3·0 g Ca/kg diet than in rats fed on the diet containing 4·5 g Ca/kg diet with or without nephrectomy. Ca excretion was higher in NPX rats than in sham rats at 3·0 g Ca/kg diet. Apparent Ca absorption was lower in the NPX groups than in the sham groups of rats fed on both sufficient (4·5 g Ca/kg) and insufficient (3·0 g Ca/kg) levels of Ca in the diet.

In NPX rats the effects of GGH ingestion and caecectomy on Ca absorption are shown in Fig. 3. Faecal Ca excretion was higher and Ca absorption was lower in CCX rats compared with non-CCX (sham) rats. In the sham group the Ca absorption rate was higher in rats fed on GGH than in rats fed on the fibre-free diet. In contrast, the absorption rate was not increased with GGH feeding in CCX rats.

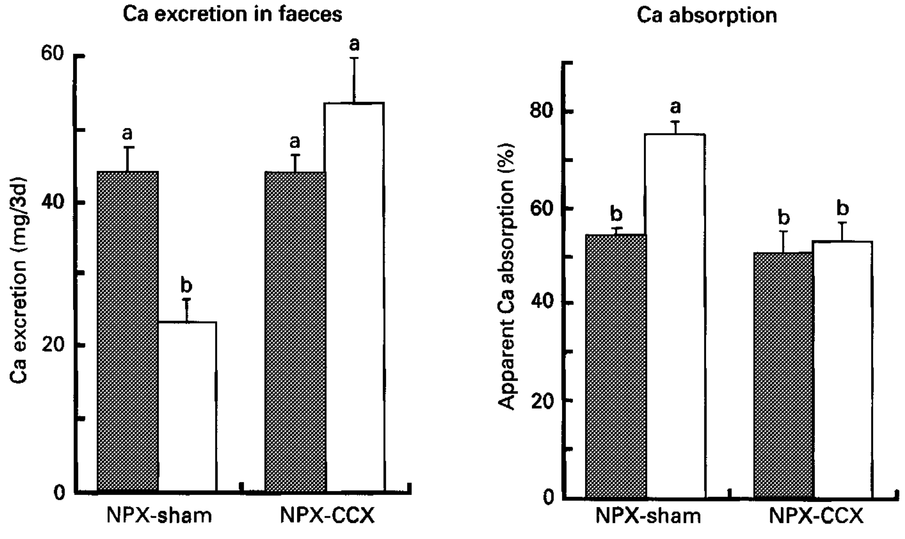


Fig. 3. Expt 3. Faecal excretion of calcium and apparent Ca absorption in partially nephrectomized (NPX) rats with caecectomy (CCX) or without (sham) after feeding a fibre-free diet (▩) or a diet containing guar-gum hydrolysate (□). All values are means with their standard errors represented by vertical bars for seven rats. The statistical significance of the effects of treatment (caecectomy; T), diet and T × diet respectively (two-way ANOVA) on faecal Ca excretion were $P = 0{\cdot}0008$, $P = 0{\cdot}1794$ and $P = 0{\cdot}0007$ and for apparent Ca absorption were $P = 0{\cdot}0009$, $P = 0{\cdot}0019$ and $P = 0{\cdot}0113$. a, b Mean values with unlike superscript letters were significantly different (Duncan's multiple-range test; $P < 0{\cdot}05$). For details of diets and experimental procedures, see Table 1 and pp. 774–776.

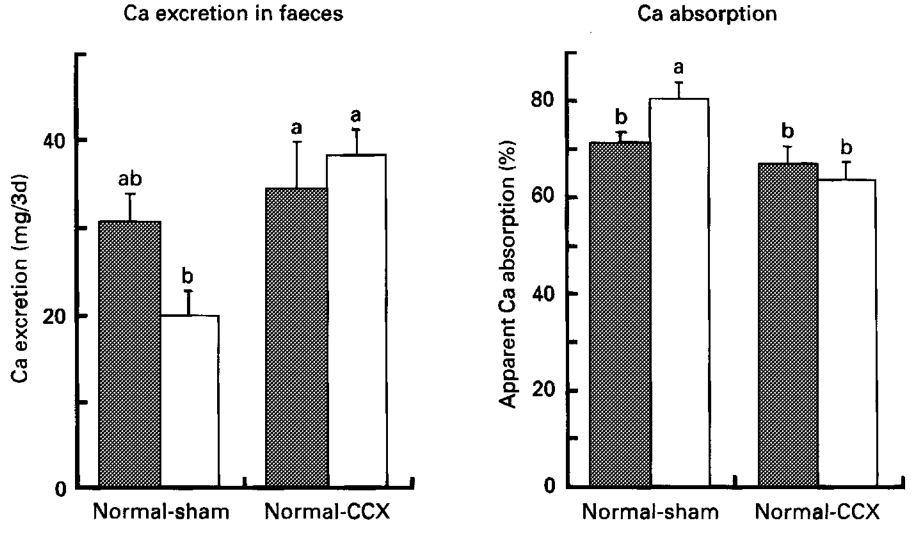


Fig. 4. Expt 4. Faecal excretion of calcium and apparent Ca absorption in normal (non-nephrectomized) rats with caecectomy (CCX) or without (sham) after feeding a fibre-free diet (▩) or a diet containing guar-gum hydrolysate (□). All values are means with their standard errors represented by vertical bars for seven rats. The statistical significance of the effects of treatment (caecectomy; T), diet and T × diet respectively (two-way ANOVA) on faecal Ca excretion were $P = 0{\cdot}0086$, $P = 0{\cdot}3833$ and $P = 0{\cdot}0659$, and for apparent Ca absorption were $P = 0{\cdot}0032$, $P = 0{\cdot}3394$ and $P = 0{\cdot}0632$. a, b Mean values with unlike superscript letters were significantly different (Duncan's multiple-range test; $P < 0{\cdot}05$). For details of diets and experimental procedures, see Table 1 and pp. 774–776.

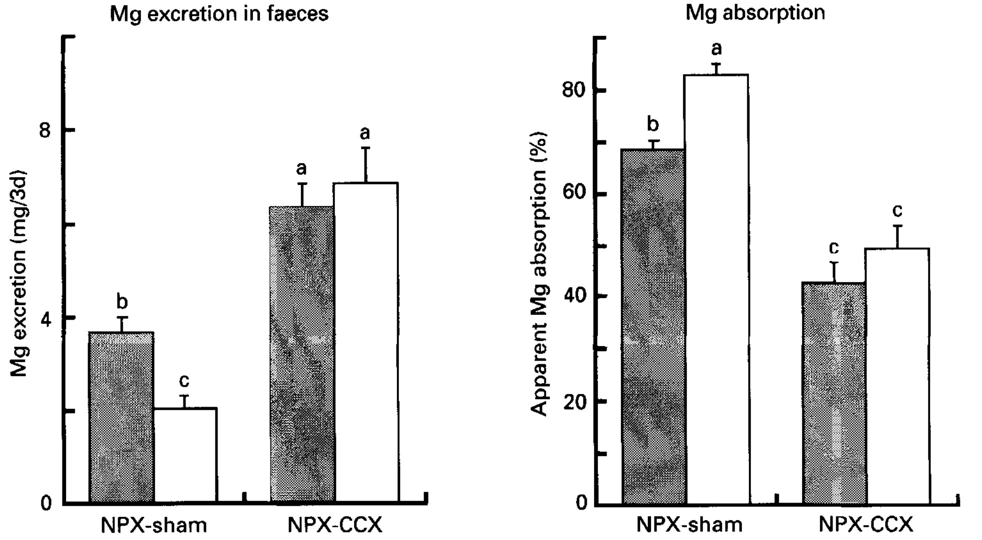


Fig. 5. Expt 3. Faecal excretion of magnesium and apparent Mg absorption in partially nephrectomized (NPX) rats with caecectomy (CCX) or without (sham) after feeding a fibre-free diet (▩) or a diet containin guar-gum hydrolysate (□). All values are means with their standard errors represented by vertical bars for seven rats. The statistical significance of the effects of treatment (caecectomy; T), diet and T × diet respectively (two-way ANOVA) on faecal Mg excretion were $P < 0{\cdot}0001$, $P = 0{\cdot}2834$ and $P = 0{\cdot}0469$ and for apparent Mg absorption were $P < 0{\cdot}0001$, $P = 0{\cdot}0038$ and $P = 0{\cdot}2836$. a, b, c Mean values with unlike superscript letters were significantly different (Duncan's multiple-range test; $P < 0{\cdot}05$). For details of diets and experimental procedures, see Table 1 and pp. 774–776.

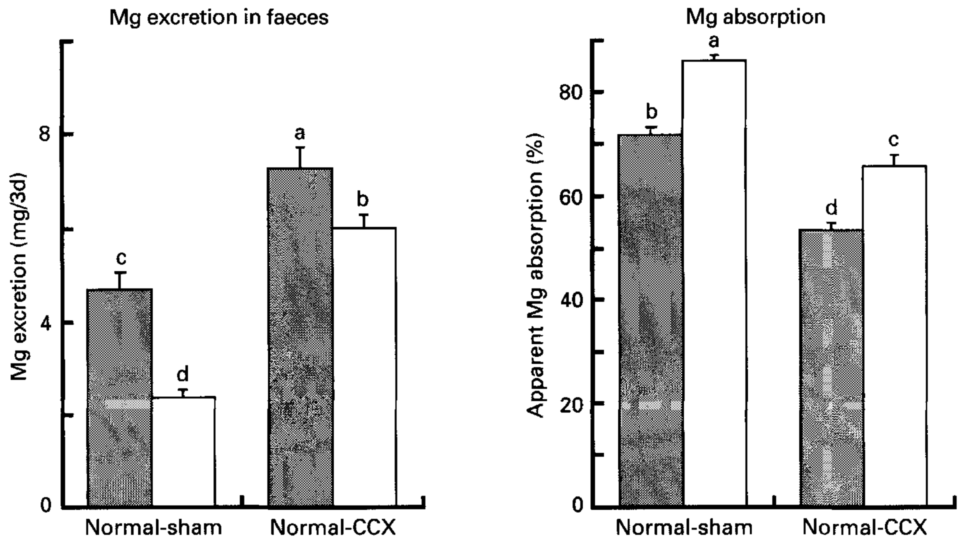


Fig. 6. Expt 4. Faecal excretion of magnesium and apparent Mg absorption in normal (non-nephrectomized) rats with caecectomy (CCX) or without (sham) after feeding a fibre-free diet (▩) or a diet containing guar-gum hydrolysate (□). All values are means with their standard errors represented by vertical bars for seven rats. The statistical significance of the effects of treatment (caecectomy; T), diet and T × diet respectively (two-way ANOVA) on faecal Mg excretion were $P < 0{\cdot}0001$, $P < 0{\cdot}0001$ and $P = 0{\cdot}4391$, and for apparent Mg absorption were $P < 0{\cdot}0001$, $P < 0{\cdot}0001$ and $P = 0{\cdot}1212$. a, b, c, d Mean values with unlike superscript letters were significantly different (Duncan's multiple-range test; $P < 0{\cdot}05$). For details of diets and experimental procedures, see Table 1 and pp. 774–776.

Table 4. *Femur calcium and magnesium contents (mg/femur) after feeding a fibre-free diet or a diet containing guar-gum hydrolysate (GGH) for 7 d to partially nephrectomized (NPX; Expt 3) and normal rats (Expt 4) with or without caecectomy (CCX or sham respectively)**

(Mean values with their standard errors for seven rats in Expt 3 and for six rats in Expt 4)

		Ca				Mg			
		NPX rats		Normal rats		NPX rats		Normal rats	
Treatment	GGH in diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sham	−	46·1[ab]	0·80	48·4[a]	1·87	0·786[a]	0·012	0·768	0·086
Sham	+	48·4[a]	0·80	51·1[a]	1·69	0·823[a]	0·017	0·801	0·069
CCX	−	44·9[b]	1·09	43·9[b]	1·07	0·671[b]	0·023	0·735	0·043
CCX	+	45·2[b]	1·41	45·2[b]	3·00	0·671[b]	0·014	0·647	0·062
Statistical significance (ANOVA) of effect of:									
Treatment (T)		$P = 0·0499$		$P = 0·0092$		$P < 0·0001$		$P = 0·1746$	
Diet		$P = 0·2081$		$P = 0·2769$		$P = 0·2988$		$P = 0·6825$	
T × diet		$P = 0·3655$		$P = 0·6799$		$P = 0·3063$		$P = 0·3723$	

[a, b] Mean values with unlike superscript letters were significantly different (Duncan's multiple-range test; $P < 0·05$).

* For details of diets and experimental procedures, see Table 1 and pp. 774–776.

Changes in Ca absorption with feeding of GGH and CCX in normal (non-NPX) rats were similar to those in NPX rats (Fig. 4). However, the increased Ca absorption with GGH ingestion for normal rats was smaller compared with that for NPX rats; i.e., average increases in Ca absorption with GGH (evaluated by subtracting the mean of Ca absorption in rats fed on the fibre-free diet from an individual value in GGH-fed rats shown in Figs 3 and 4) were +21·3% (*n* 7) in the NPX group and +9·31% (*n* 6) in the normal group ($P = 0·0149$).

The changes in Mg absorption in NPX and normal (non-NPX) rats (Figs 5 and 6 respectively) were similar to those of Ca absorption; ingestion of GGH increased apparent Mg absorption in sham (non-CCX) rats, but not in CCX rats. However, in both NPX and non-NPX rats fed on the fibre-free diet, caecectomy decreased Mg absorption, especially in NPX rats.

Table 4 shows that the amount of femur Ca was significantly lower in CCX rats than in non-CCX rats in the NPX and non-NPX groups (ANOVA; treatment). Diet did not influence femur Ca and Mg (two-way ANOVA).

DISCUSSION

The present study shows that apparent Ca absorption lowered by partial nephrectomy (NPX rats) was increased by feeding a soluble dietary fibre, GGH (Fig. 3). Feeding of GGH also caused small but significant increases in Ca absorption in normal rats (Fig. 4). These increments in Ca absorption with feeding of GGH were completely abolished by caecectomy (Figs 3 and 4). These findings reveal that enhancements of Ca absorption with feeding of GGH depend on the caecum and that a considerable amount of Ca was absorbed from the large intestine in GGH-fed rats. In contrast, Ca absorption in the fibre-free groups was not influenced by caecectomy in NPX and normal rats (Figs 3 and 4). The results show that Ca absorption in the large intestine is negligible in rats fed on a fibre-free diet, which agrees with results of previous reports that the major site of Ca absorption is the small intestine (Marcus & Lengemann, 1962; Hurwitz & Bar, 1966; Annaka *et al.* 1989).

We adopted five-sixths partially nephrectomized rats as a model of renal failure; the treatment reduces renal function quantitatively. In the model of renal failure, vitamin D hydroxylation is decreased (Kawashima & Kurokawa, 1983) and intestinal active transport of Ca is decreased (Walling *et al.* 1976; Schiffl & Binswanger, 1980; Koller & Binswanger, 1982). In the present study, Ca absorption was clearly lower in NPX rats than in intact (sham) rats even at an insufficient level of Ca (3·0 g/kg) in diet (Fig. 2). Comparison between the NPX-sham (non-CCX) and normal-sham groups (Figs 3 and 4), shows that Ca absorption in NPX rats was considerably lower than that in normal rats in the fibre-free groups ($-17{\cdot}1\,\%$, $P < 0{\cdot}0001$), but the absorption in fibre-fed NPX rats was slightly lower than that in normal fibre-fed rats ($-5{\cdot}11\,\%$, $P = 0{\cdot}2438$). These findings suggest that nephrectomy does not influence the absorption of Ca in the large intestine induced by fibre feeding, and the increase in the caeco-colonic absorption compensates the decreasing proximal intestinal Ca transport associated with nephrectomy. In an *in vitro* study, Karbach & Feldmeier (1993) showed that the caecum and the proximal colon have the highest absorptive capacity, and the high absorptive capacity in the large intestine is associated with the vitamin D-independent paracellular pathway of Ca transport. These results support our suggestion.

A possible mechanism for the increase in the caeco-colonic Ca absorption associated with feeding GGH is luminal acidification resulting from caecal fermentation of dietary fibre, and an increase in ionic Ca in the large intestine. In the present study the stimulatory effects on Ca absorption of feeding GGH were abolished with resection of the caecum, in which dietary fibre is mainly fermented and organic acids are produced. Table 3 shows that caecectomy increased faecal dry weight of fibre-fed rats by 1·7–1·8 g/3 d, which is comparable with the amounts of GGH ingested for 3 d, whereas faecal dry weight of non-CCX rats fed on GGH was not increased compared with those of rats fed on a fibre-free diet. These results demonstrate that almost all ingested GGH was fermented in the caecum and the fermentation was absent in CCX rats. Previously, Takahashi *et al.* (1994*a*, *b*) reported a higher concentration of short-chain fatty acids and lower pH in the caecum in rats fed on a diet containing GGH compared with rats fed on a fibre-free diet.

Apparent Mg absorption increased with feeding of GGH and decreased with caecectomy, similarly to Ca absorption (Figs 5 and 6), which suggests that the large intestine contributes to Mg absorption. However, even in the fibre-free groups, Mg absorption was decreased with caecectomy in both normal and NPX rats (Figs 5 and 6). Ohta *et al.* (1994) shows similar results to those of the present study in caecectomized rats fed on slightly-digestible oligosaccharide. These results show that Mg was absorbed in the large intestine not only in fibre-fed rats but also in rats fed on a fibre-free diet. Chutkow (1964, 1966) reported that Mg is predominantly absorbed in the large intestine.

In Expts 3 and 4 (effects of CCX and GGH), coprophagy was prevented so that the effects of caecectomy on Ca and Mg absorption could be examined. Prevention of coprophagy confirmed that small intestinal absorption of re-ingested Ca in faeces was not involved in the increases in Ca absorption with feeding of GGH. In the present study, for normal rats fed on a fibre-free diet apparent Ca absorption in the absence of coprophagy (71·3 %; sham, fibre-free in Fig. 4) was somewhat lower than that when coprophagy was allowed (84·0 %; 3·0 g/kg, sham in Fig. 2, $P = 0{\cdot}0060$). Cree *et al.* (1986) reported that prevention of coprophagy with a restraining cage strikingly decreased Ca absorption. The differences from our results may be due to the degrees of physical and psychological stress.

The amount of femur Ca was decreased with caecectomy ($P = 0{\cdot}0499$ in NPX rats and $P = 0{\cdot}0092$ in normal rats), as shown in Table 4. Fig. 1 shows that the relationship between the amount of Ca absorbed and the Ca content of the femur was linear in rats fed on a Ca-deficient diet. The reduction in femur Ca with caecectomy implies that the colon affects bone Ca metabolism through intestinal absorption. Femur Ca did not change significantly with feeding GGH and with nephrectomy (Table 4), but this may have been because the experimental period (7–10 d) was too short for changes in Ca absorption to be reflected in bone Ca.

In conclusion, feeding a highly-fermentable soluble dietary fibre induced caeco-colonic absorption of Ca, which compensates for lowered absorption of Ca associated with renal failure.

REFERENCES

American Institute of Nutrition (1977). Report of the American Institute of Nutrition *ad hoc* Committee on Standards for Nutritional Studies. *Journal of Nutrition* **107**, 1340–1348.

American Institute of Nutrition (1980). Second report of the *ad hoc* Committee on Standards for Nutritional Studies. *Journal of Nutrition* **110**, 1726.

Annaka, A., Watanabe, E., Sunahara, T. & Ishibashi, T. (1989). Site and rate of absorption and secretion of calcium and phosphorus in laying hens. *Bulletin of the Faculty of Agriculture Niigata University* **41**, 37–44.

Chutkow, J. G. (1964). Sites of magnesium absorption and excretion in the intestinal tract of the rat. *Journal of Laboratory and Clinical Medicine* **63**, 71–79.

Chutkow, J. G. (1966). Effect of magnesium deficiency on location of the intestinal absorption of magnesium in rats. *Proceedings of the Society for Experimental Biology and Medicine* **123**, 836–840.

Coburn, J. W., Koppel, M. H., Brickman, A. S. & Massry, S. G. (1973). Study of intestinal absorption of calcium in patients with renal failure. *Kidney International* **3**, 264–272.

Cree, T. C., Wadley, D. M. & Marlett, J. A. (1986). Effect of preventing coprophagy in the rats on neutral detergent fiber digestibility and apparent calcium absorption. *Journal of Nutrition* **116**, 1204–1208.

Démigné, C., Levrat, M. & Rémésy, C. (1989). Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between the cecum and blood plasma in the rat. *Journal of Nutrition* **119**, 1625–1630.

Démigné, C. & Rémésy, C. (1985). Stimulation of absorption of volatile fatty acids and minerals in the cecum of rats adapted to a very high fiber diet. *Journal of Nutrition* **115**, 53–60.

Donangelo, C. M. & Eggum, B. O. (1986). Comparative effects of wheat bran and barley husk on nutrient utilization in rats. *British Journal of Nutrition* **56**, 269–280.

Harper, A. E. (1959). Amino acid balance and imbalance. 1. Dietary level of protein and amino acid imbalance. *Journal of Nutrition* **68**, 405–418.

Hurwitz, S. & Bar, A. (1966). Rate of passage of calcium-45 and Yttrium-91 along the intestine, and calcium absorption in the laying fowl. *Journal of Nutrition* **89**, 311–316.

Karbach, U. & Feldmeier, H. (1993). The cecum is the site with the highest calcium absorption in rat intestine. *Digestive Diseases and Sciences* **38**, 1815–1824.

Kawashima, H. & Kurokawa, K. (1983). Unique hormonal regulation of vitamin D metabolism in the mammalian kidney. *Mineral and Electrolyte Metabolism* **9**, 227–235.

Kaye, M. & Silverman, M. (1965). Calcium metabolism in chronic renal failure. *Journal of Laboratory and Clinical Medicine* **66**, 535–548.

Koller, M. & Binswanger, U. (1982). Calcium transport in the ileum of uremic rats. *American Journal of Physiology* **242**, G128–G134.

Marcus, C. S. & Lengemann, F. W. (1962). Absorption of Ca^{45} and Sr^{85} from solid and liquid food at various levels of the alimentary tract of the rat. *Journal of Nutrition* **77**, 155–160.

Morrison, A. B. (1962). Experimentally induced chronic renal insufficiency in the rat. *Laboratory Investigation* **11**, 321–332.

Ohta, A., Baba, S., Ohtsuki, M., Taguchi, A., Adachi, T. & Hara, H. (1996). Prevention of coprophagy modifies magnesium absorption in rats fed with fructo-oligosaccharides. *British Journal of Nutrition* **75**, 775–784.

Ohta, A., Ohtuki, M., Takizawa, T., Inaba, H., Adachi, T. & Kimura, S. (1994). Effects of fructooligosaccharides on the absorption of magnesium and calcium by cecectomized rats. *International Journal of Vitamin and Nutrition Research* **64**, 316–323.

Oku, T., Konishi, F. & Hosoya, N. (1982). Mechanism of inhibitory effect of unavailable carbohydrate on the intestinal calcium absorption. *Journal of Nutrition* **112**, 410–415.

Reeves, P. G. (1989). AIN-76 diet: should we change the formulation? *Journal of Nutrition* **119**, 1081–1082.

Shiffl, H. & Binswanger, U. (1980). Calcium ATPase and intestinal calcium transport in uremic rats. *American Journal of Physiology* **238**, G424–G428.

Statistical Analysis Systems (1989). *SAS User's Guide, Statistics. Release* 6.0.8. Cary, NC; SAS Institute Inc.

Takahashi, H., Yang, S. I., Kim, M. & Yamamoto, T. (1994*a*). Protein and energy utilization of growing rats fed on the diets containing intact or partially hydrolyzed guar gum. *Comparative Biochemistry and Physiology* **107**A, 255–260.
Takahashi, H., Yang, S. I., Ueda, Y., Kim, M. & Yamamoto, T. (1994*b*). Influence of intact and partially hydrolyzed guar gum on iron utilization in rats fed on iron-deficient diets. *Comparative Biochemistry and Physiology* **109**A, 75–82.
van der Aar, P. J., Fahey, G. C. Jr, Ricke, S. C., Allen, S. E. & Berger, L. L. (1983). Effects of dietary fibers on mineral status of chicks. *Journal of Nutrition* **113**, 653–661.
Walling, M. W., Kimberg, D. V., Wasserman, R. H. & Feinberg, R. R. (1976). Duodenal active transport of calcium and phosphate in vitamin D-deficient rats: effects of nephrectomy *Cestrum diurnum*, and 1α,25-dihydroxy vitamin D_3. *Endocrinology* **98**, 1130–1134.

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