

Passive uptake in the small intestine and active uptake in the hindgut contribute to the highly efficient mineral metabolism of the common mole-rat, *Cryptomys hottentotus*

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Cryptomys hottentotus has no access to dietary or endogenous sources of cholecalciferol (D_3). Serum concentrations of calcifediol ($25(OH)D_3$) were undetectable (< 5 ng/ml) and calcitriol ($1,25(OH)_2D_3$), although detectable in plasma, was at a low concentration (31.40 (SEM 3.06) pg/ml). Despite their naturally impoverished vitamin D status, these animals exhibited highly efficient mineral absorption processes (Ca apparent fractional absorption efficiency, AFA (%) 95.33 (SEM 1.36); inorganic P (P_i) AFA (%) 93.49 (SEM 0.81)). Furthermore, plasma mineral content was tightly regulated (Ca 2.57 (SEM 0.08) mmol/l; Mg 1.23 (SEM 0.05) mmol/l; P_i 2.12 (SEM 0.15) mmol/l). Mode of uptake in *C. hottentotus* was unlike that in other D_3 -replete mammals. First, passive (rather than active) uptake occurred in the traditional site of active Ca absorption (with serosal:mucosal (S:M) ratios in the duodenum of 1.32 (SEM 0.13)), and the only site of active Ca uptake was the hindgut (caecum S:M 3.35 (SEM 0.46); proximal colon S:M 2.13 (SEM 0.30)). Despite the presence of active uptake in these hindgut regions, their overall contribution to the daily rate of mineral absorption was small (9.53 (SEM 1.27) %). These underground inhabitants rely upon highly efficient, passive mineral uptake. This is adequate to meet their mineral requirements and maintain mineral homeostasis in the absence of vitamin D.

Mineral metabolism: Calcium: Mole-rat

The common mole-rat, *Cryptomys hottentotus* (order Rodentia; family *Bathyergidae*), lives underground in an extensive maze of tightly plugged burrows, in the mesic areas of southern and central Africa (de Graaff, 1981). Given their strictly subterranean, chthonic habitat and herbivorous diet, these animals have no obvious natural source of cholecalciferol (D_3). In addition, Ca requirements in mole-rats are high as their large, evergrowing incisors are continually worn down during burrow excavations and replaced. It is speculated, therefore, that the common mole-rat is deficient in D_3 and might meet its mineral requirements in a unique D_3 -independent manner. Furthermore, as this animal is a hindgut fermenter, the site of nutrient absorption might be lower down the gut than commonly reported (duodenum).

Previous mineral studies that addressed the problem of mineral metabolism in chthonic inhabitants (*Cryptomys damarensis*, Skinner *et al.* 1991; Pitcher *et al.* 1992, and *Heterocephalus glaber*, Buffenstein & Yahav, 1991*a*) indicate that dietary Ca absorption is mediated through highly efficient non-saturable processes. These chthonic species absorbed more than 85% of the available Ca in their diet (Buffenstein & Yahav, 1991*a*; Skinner *et al.* 1991; Pitcher *et al.* 1992), whereas the efficiency of most other mammals is below 60% (Brommage & Baxter, 1988*a*; Hughes, 1988). It is not known whether these characteristics are typical of all subterranean mammals. Furthermore, the mechanisms employed are not fully understood. We addressed these questions first by monitoring mineral homeostasis in

freshly caught *C. hottentotus*, fed a diet similar in Ca content to their natural diet, then by examining the mode of Ca uptake along the entire length of the gastrointestinal tract (GIT).

MATERIALS AND METHODS

Animals and management

Freshly caught male and female adult animals (112.89 (SEM 12.05) g) were used in this study. Animals were trapped on the Witwatersrand, Johannesburg, South Africa. Thereafter, they were housed in the Central Animal Unit (University of the Witwatersrand Medical School) under constant climatic conditions (ambient temperature $26 \pm 2^\circ$ and humidity 60%) in a darkened room supplied with a single incandescent (40 W) light bulb. They were kept in colonies in glass terraria and transparent perspex burrow systems which were partially filled with vermiculite. Before experimentation, all animals were given an *ad. lib.* diet of sweet potato (*Ipomoea batatas*) and apple. Mole-rats adapted well to captivity, remaining healthy and maintaining body mass.

Experimental treatment

The animals were placed on a diet of sweet potato (containing (mg/g) Ca 1.7, inorganic P (P_i) 2.9, water 753; 15.84 kJ/g dry weight) and apple (containing (mg/g) Ca 0.5, P_i 0.8, water 855; 16.43 kJ/g dry weight) for at least 4 weeks before experimentation, to enable the gut microfauna to adapt (if need be) to the change in diet (Buffenstein & Yahav, 1991*b*). During the experimental period they were housed individually in metabolic cages (Techniplast, Johannesburg), facilitating the measurement of mineral balance and collection of leftover food and of urine and faeces. The quantity of food eaten and urine and faeces produced was monitored over an 11 d period.

Representative food samples, uneaten food, and faeces were weighed and dried to a constant weight. Glass-fibre filter papers (Whatman GF/D) were placed over the openings of the urine collection tubes to prevent contamination. Urine was collected under light liquid paraffin and measured to an accuracy of 0.1 ml. A small drop of acetic acid was placed in each urine collection tube to prevent bacterial proliferation. Representative samples of urine were frozen at -70° .

Plasma and tissue collection

All animals were anaesthetised with Sagatal (30 mg/kg). Thereafter, they were killed by cardiac exsanguination. Blood samples collected during this procedure were centrifuged and the plasma separated and frozen at -70° for later analyses.

Plasma analyses included measurement of Ca (ionized Ca (Ca^{2+}) and total Ca), P_i , Mg and the vitamin D metabolites calcifediol (25(OH) D_3) and calcitriol (1,25(OH) $_2D_3$).

Teeth and bones collected at the end of the experimental period were stored at -20° for later mineral analyses. The meat was removed from the bones by Dermestid beetles (courtesy of Mrs Lemma, SABS, Pretoria, South Africa). Bones were ashed overnight at 175° and dissolved in 2 ml concentrated HCl.

Vitamin D metabolites

Serum total 25(OH) D_3 and 1,25(OH) $_2D_3$ metabolite concentrations were measured using the methods described by Haddad & Chyu (1971) and Reinhardt *et al.* (1984) respectively. Before metabolite determination, plasma samples were subjected to extraction and purification techniques as described by Turnbull *et al.* (1982). The sensitivities of the 25(OH) D_3 and 1,25(OH) $_2D_3$ assays were 5 ng/ml and 4 pg/ml serum respectively.

Table 1. *Body mass, food intake and food digestibility in the mole-rat (Cryptomys hottentotus) fed on sweet potato (Ipomoea batatas) and apple*

Mean values with their standard errors for eight animals)

	Mean	SEM
Body mass (g)	112.89	12.05
Increase in body mass (%/11 d)	8.09	2.56
Food intake (g DM/100 g body wt per d)	8.20	0.68
AFA (%)		
Ca	95.33	1.36
P _i	93.49	0.81
AFR (%)		
Ca	97.79	0.49
P _i	94.49	2.67

AFA, apparent fractional absorption; AFR, apparent fractional retention; P_i, inorganic phosphorus.*Mineral analyses*

Ca and Mg in plasma, food, faeces, urine, bone and teeth was determined in a solution of LaCl₃ (2 g La/l) by atomic absorption spectrophotometry (Varian Spectra 10). P_i was assayed colorimetrically using a commercially available kit based on the method of Hurst (1967) and Kraml (1966). Apparent fractional absorption (AFA) was calculated as

$$\text{AFA} = \frac{(X \text{ ingested/d}) - (X \text{ in faeces/d})}{(X \text{ ingested/d})} \times 100,$$

where X is Ca, P_i or Mg. The apparent fractional retention (AFR) was similarly calculated

$$\text{AFR} = \frac{(X \text{ absorbed/d}) - (X \text{ in urine/d})}{(X \text{ absorbed/d})} \times 100$$

where X is Ca, P_i or Mg.

Alkaline phosphatase

Plasma alkaline phosphatase (EC 3.1.3.1) was assayed colorimetrically (Timepac alk phos reagent; Technicon No: T40-0009), based on the methods described by Morgenstein *et al.* (1965).

Mode of calcium uptake

On completion of the mineral balance study, mode of uptake of Ca was determined using a modified everted gut sac technique (as described by Wilson & Wiseman, 1954). Briefly, mole-rats were anaesthetised with Sagatal (30 mg/kg) and an incision made along the ventral midline. Thereafter, the intestine was carefully excised and placed into a well-oxygenated (O₂-CO₂; 95:5; v/v) storage solution (146 mM-NaCl; 3.99 mM-KCl).

The intestine was divided into five sections: duodenum (i.e. from the pyloric sphincter to the ligament of Treitz), jejunum, proximal-, mid-, and distal ileal sections (Wilson & Wiseman, 1954). In addition, the caecum, proximal- and distal colon were removed and thoroughly rinsed with the storage solution. Everted gut sacs of each of these sections were made using 30–35 mm lengths of intestine. They were filled with a known volume of buffered solution, comprising 151.1 mM-NaCl, 20 mM-D + glucose, 0.299 mM-CaCl₂ · 2H₂O,

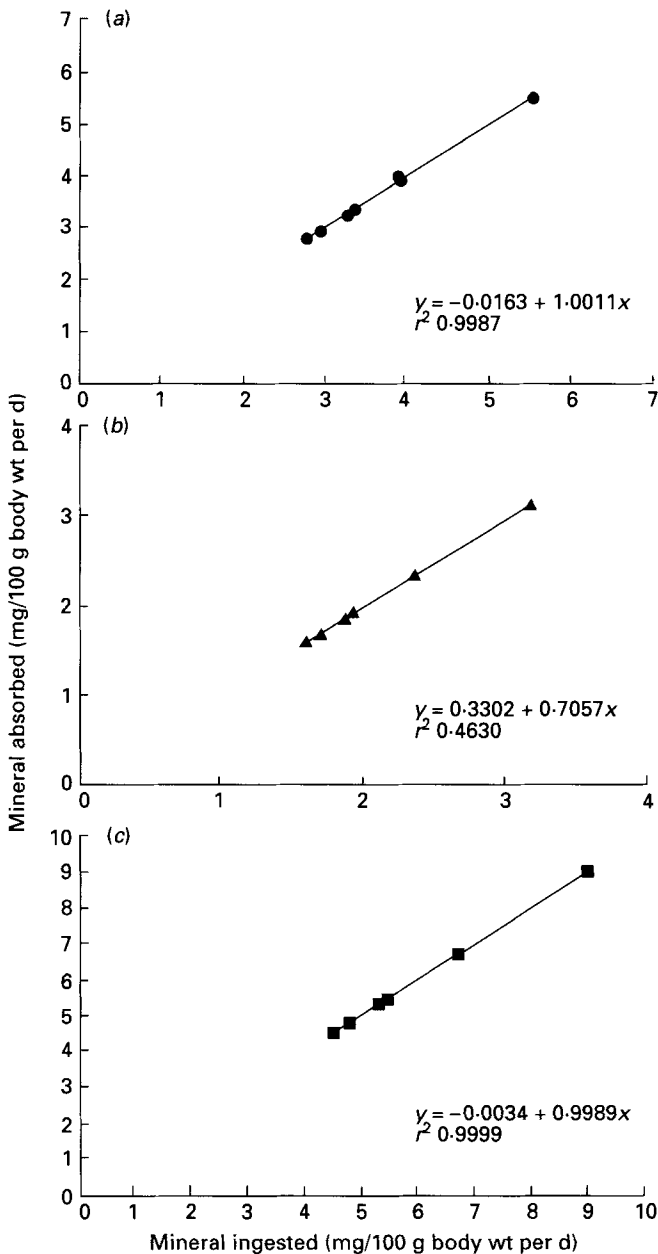


Fig. 1. Apparent absorption of (a) calcium, (b) magnesium and (c) inorganic phosphorus by the mole-rat (*C. hottentotus*), showing direct dependence on the amount of mineral ingested. The points indicate individual animals (n 8).

3.219 mM- Na_2HPO_4 and 0.693 mM- $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, to which ^{45}Ca (code no. CES 3; Amersham) was added. The intestinal sacs were incubated in the same buffer solution, and continually oxygenated (O_2 - CO_2 ; 95:5; v/v) for a period of 3 h. Thereafter, the gut sacs were removed and the fluid inside the gut sacs drained into test tubes. Volume changes were measured and the concentration of ^{45}Ca was determined using a Packard Tri-carb (model

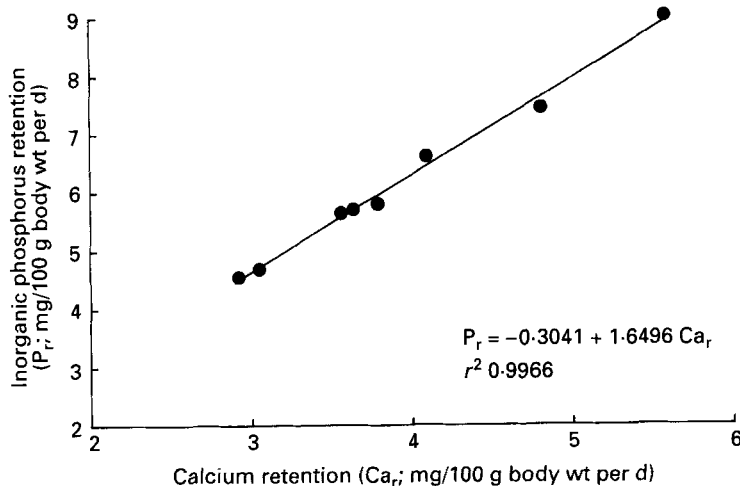


Fig. 2. The relationship between calcium retention and inorganic phosphorus retention for the mole-rat (*C. hottentotus*). The points indicate individual animals (n 8).

No. 300C) scintillation counter. All data, after correcting for volume changes, were expressed as the S:M ratio, i.e. final concentration of ^{45}Ca inside the intestinal sac (serosal; S):the concentration of ^{45}Ca outside the sac (mucosal; M). S:M ratios > 1.5 were considered indicative of active transport.

Statistical analyses

All data are expressed as means with their standard errors. Linear regression analyses were determined in accordance with the methods of Zar (1974).

RESULTS

Freshly caught experimental animals supplied with food *ad. lib.* gained about 0.7% body weight/d over the 11 d experimental period (Table 1).

Mineral balance

The AFA (%) of Ca and P_i for the sweet potato and apple was exceptionally high (Table 1), with a significant correlation between the amount ingested and the amount absorbed for Ca, Mg and P_i (Fig. 1). There was, however, no correlation between the amount of each element absorbed and the amount excreted in the urine.

Mineral balance was in a state of positive flux for both elements, with Ca showing the higher AFR and that of P_i being approximately 3% less (Table 1). A highly significant ($P < 0.001$) linear relationship existed between Ca retention and P_i retention (Fig. 2).

Bone and teeth mineral data show that there was significantly more Ca and P_i present in the teeth (per mm) of *C. hottentotus* than in the bones (Table 2). Teeth have an average length of 16.17 ± 0.64 mm.

Plasma mineral and D_3 metabolite concentrations

Plasma $25(\text{OH})D_3$ levels were below the sensitivity of the assay (< 5 ng/ml; Table 3), whereas $1,25(\text{OH})_2D_3$ was present (Table 3). Plasma mineral concentrations were tightly regulated and within the normal range for mammals (Table 3).

Table 2. Length of teeth and mineral contents of teeth and bones from the mole-rat (*Cryptomys hottentotus*)

(Mean values with their standard errors for eight animals)

	Teeth		Long bones	
	Mean	SEM	Mean	SEM
Length (mm)	16.17	0.64		
Ca (mmol/g)	4.58	0.36	2.40	0.07
P _i (mmol/g)	3.32	0.25	2.20	0.43

P_i, inorganic phosphorus.Table 3. Plasma mineral and vitamin D metabolite concentrations of the mole-rat (*Cryptomys hottentotus*)

(Mean values with their standard errors for eight animals)

	Mean	SEM
Ionized Ca (mmol/l)	1.38	0.01
Total Ca (mmol/l)	2.57	0.08
Mg Ca (mmol/l)	1.23	0.05
P _i (mmol/l)	2.12	0.15
Alkaline phosphatase (EC 3.1.3.1; IU/l)	266.40	29.40
25(OH)D ₃ (ng/ml)	< 5	
1,25(OH) ₂ D ₃ (pg/ml)	31.40	3.06

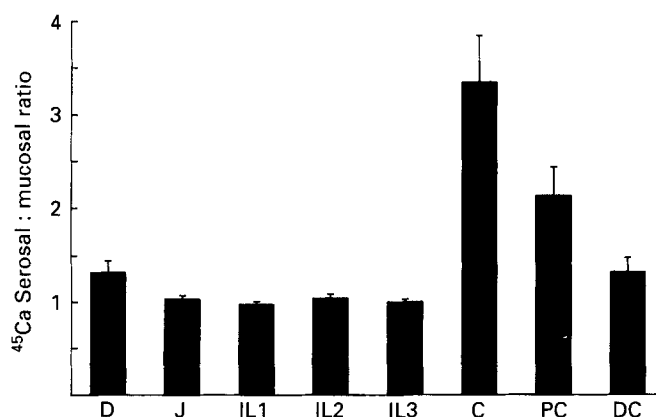
P_i, inorganic phosphorus.

Fig. 3. Serosal : mucosal (S : M) ratios as indicators of active and passive transport along the entire gastrointestinal tract of the mole-rat (*C. hottentotus*). D, duodenum; J, jejunum; IL1, ileal segment 1; IL2, ileal segment 2; IL3, ileal segment 3; C, caecum; PC, proximal colon; DC, distal colon. S : M > 1.5 indicates active uptake; S : M < 1.5 indicates passive uptake. Values are means with their standard errors.

⁴⁵Ca uptake

Active uptake of ⁴⁵Ca in *C. hottentotus* occurred in the hindgut only (caecum S : M 3.35 (SEM 0.46); proximal colon S : M 2.13 (SEM 0.30)), whereas along the entire length of the small intestine the uptake of ⁴⁵Ca was passive (duodenum S : M 1.32 (SEM 0.13); Fig. 3).

DISCUSSION

Mineral homeostasis (Table 1) and vitamin D status (Table 3) in *C. hottentotus* were similar to that of other mole-rat species (Buffenstein & Yahav, 1991*a*; Skinner *et al.* 1991; Pitcher *et al.* 1992), in that animals exhibited a positive net flux and highly efficient mineral absorption occurred despite an apparently impoverished vitamin D status. Furthermore, mode of uptake in the small intestine was similarly found to be passive (Fig. 3; Pitcher *et al.* 1992). This is, however, the first report to date of active uptake in the hindgut, rather than the traditional site of active Ca absorption, the duodenum.

Plasma metabolites

Serum levels of the principal circulating form of vitamin D (25(OH)D₃) are generally indicative of D₃ status (Audran & Kumar, 1985). An undetectable level of 25(OH)D₃ (Table 3) in *C. hottentotus* suggests that these animals have an impoverished vitamin D status. Whilst the active metabolite is present (Table 3) in mole-rat plasma, it is in much lower amounts than that previously reported for other rodents (Clark *et al.* 1987). This again may reflect natural D₃ deficiency. The low plasma concentrations present, however, appear sufficient to maintain normal plasma mineral concentrations (Table 3), a positive mineral flux (Table 1) and mineral homeostasis. The positive mineral balance is of no pathological consequence as excess mineral is deposited in the evergrowing and worn down teeth (Table 2).

Wild wood mice, bank voles (Shore *et al.* 1988) and horses also show detectable levels of 1,25(OH)₂D₃ in the absence of 25(OH)D₃. It is suggested that they do not utilize vitamin D-mediated intestinal absorption (Horst *et al.* 1988). Rather, passive Ca absorption may prove sufficient for their mineral needs (Shore *et al.* 1988). This hypothesis, although untested and currently pure speculation, appears to hold true for *C. hottentotus* and other mole-rats, all of which maintain mineral homeostasis despite undetectable 25(OH)D₃ concentrations (Buffenstein & Yahav, 1991*a*; Pitcher *et al.* 1992; Skinner *et al.* 1991), and all of which show passive duodenal mineral absorption.

Mineral homeostasis

The AFA(%) of *C. hottentotus* parallels that of the other species of mole-rats studied to date, *C. damarensis* (Skinner *et al.* 1991; Pitcher *et al.* 1992) and *H. glaber* (Buffenstein & Yahav, 1991*a*), in that AFA exceeds 85%. In this study, despite the observed apparent impoverished vitamin D status, absorption of Ca is extremely efficient (95.33 (SEM 1.36)%), and is far higher than that previously reported in adult, non-breeding D₃-replete mammals (30–70%; Schaafsma *et al.* 1985; Brommage & Baxter, 1988*a*; Silverberg, 1990). In addition, there is a significant correlation between the amount of each element ingested and the amount absorbed (Fig. 1). Mineral homeostasis appears, therefore, to occur via a highly efficient non-saturable mechanism. Similar non-saturable D₃-independent modes of mineral uptake have been reported in rats in which unique demands are placed on both Ca homeostasis and skeletal metabolism (Dostal & Toverud, 1984; Brommage & Baxter, 1988*b*), and in other species of mole-rat studied to date (Buffenstein & Yahav, 1991*a*; Skinner *et al.* 1991; Pitcher *et al.* 1992).

There is no correlation between the amount of each element absorbed and the amount excreted in the urine; however, the retention of P_i is closely correlated to Ca retention (Fig. 2; $P_r = -0.3041 + 1.6496 Ca_r$, $r^2 0.9966$; where P_r and Ca_r are P_i and Ca retention (mg/100 g body weight per d) respectively). P_i retention, like that of other hindgut fermenters (Braithwaite, 1975), appears, therefore, to be linked to that of Ca retention. Metabolism of P_i is closely related to Ca metabolism in D₃-deficient animals, with a low-

Ca diet resulting in an increase in parathyroid hormone (PTH) and a concomitant increase in urinary P_i excretion (Braithwaite, 1975). This holds true for *C. hottentotus*, where P_i AFR (94.49%) was lower than that of Ca (97.79%).

An alternative explanation for the higher P_i urinary loss compared with Ca is afforded by the Ca: P_i ratio of the teeth. The Ca: P_i ratio for *C. hottentotus* teeth is 1.36 (SEM 0.06), which indicates that proportionately more Ca than P_i is deposited in the teeth. This difference in the Ca: P_i ratio in the teeth may explain the greater loss of P_i than Ca through urinary excretion (Table 1).

The Ca: P_i retention ratio in mole-rats was 1.226. This correlates well with the Ca: P_i ratio (1:1.25) suggested by Thomas *et al.* (1988) as corresponding to the proportion of Ca and P_i needed by rodents for whole body growth.

Uptake of ^{45}Ca along the entire GIT

C. hottentotus exhibited a most unusual mode of mineral uptake in the GIT, in that uptake was passive in the traditional site of active Ca absorption (the duodenum), and active in the hindgut, a region not normally associated with mineral absorption (Petith & Schedl, 1976).

Active gastrointestinal uptake is regulated by vitamin D (Norman, 1990). Usually in the absence of D_3 , Ca uptake in the duodenum is passive and inadequate for normal physiological function. Young, vitamin D-deficient rats do not conform to this generalization, in that they may employ active uptake in the duodenum despite their deficient D_3 status, but show no GIT active uptake elsewhere (Walling *et al.* 1974). Mole-rats, despite their D_3 -deficient status, employ active mineral uptake in the caecum and proximal colon, sites not previously known to be involved with mineral uptake. Previous mole-rat studies in our laboratory did not examine the hindgut as a potential site for active Ca uptake. It is, therefore, possible that caecal active transport is used by all mole-rat species.

The employment of active transport in the hindgut, rather than the small intestine, might be an adaptation to the hindgut location of a fermentation chamber. Fermentation of fibre will liberate mineral previously trapped within the plant fibres. If this liberated mineral is to be retained in the body, it must be rapidly absorbed before faecal formation. Detection of active transport in the caecum could reflect vitamin D-mediated pathways at the low plasma concentrations of active hormone. Caecal function, and fermentation in particular, is influenced by vitamin D supplementation to mole-rats (Buffenstein & Yahav, 1991b). This in turn implies that low hormonal levels may function in this region (a) to improve mineral uptake and (b) to assist in fermentative micro-organism proliferation and function, although caecal active uptake might be mediated by vitamin D-independent pathways.

The caecum and proximal colon show high S:M ratios of 3.35 (SEM 0.46) and 2.13 (SEM 0.30) respectively, clearly indicative of active transport in these regions. The actual contribution of active transport to the total amount of mineral absorbed (mg/100 g body weight per d) was determined by calculating the amount of Ca that is actively absorbed in the caecum and proximal colon, and correcting this to a daily rate. This was then compared with the total daily net fractional absorption. Active transport accounted for less than 10% of the net absorption. The majority of Ca absorbed must therefore occur via a non-saturable, passive process. This hypothesis is supported by the consistently high mineral absorption efficiency data in the absence of vitamin D supplementation (Buffenstein & Yahav, 1991a; Skinner *et al.* 1991; Pitcher *et al.* 1992). Intestinal Ca absorption, although not apparently regulated by vitamin D, is therefore obviously sufficient to maintain normal plasma homeostasis.

In hindgut fermenters, nutrients trapped within cell walls and fibre may be released only after fermentation in the caecum is complete. It is not known whether all hindgut

fermenters, including man, increase absorption of nutrients in the hindgut if fed on a high-fibre diet. Although most commercially important domestic animals are foregut fermenters and would therefore absorb nutrients adequately from the duodenum and not need this additional site for nutrient absorption, active absorption in the hindgut could be beneficial to non-ruminant herbivores e.g. horses (Schryver *et al.* 1970). It would be most advantageous for arid-adapted animals and animals eating a mineral-deficient diet to employ such a hindgut mechanism in order to extract fully most of the available nutrients and minerals.

In conclusion, *C. hottentotus* employs a different mode of mineral uptake to that of most mammals, with highly efficient passive transport in the duodenum and active uptake in the caecum and proximal hindgut. These regions of active mineral uptake are traditionally associated with symbiotic micro-organism fibre digestion. Employment of active transport here could act as a mineral trap. This would ensure that any mineral released during the fermentation process is absorbed for use by the animal, rather than lost in the faeces. It is most likely that caecal uptake plays a secondary role to passive duodenal transport, and merely facilitates the absorption of small quantities of mineral that have escaped prior absorption. Even in the absence of adequate amounts of vitamin D, passive transport is sufficient for mineral homeostasis.

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