Adaptations in the calcium and phosphorus metabolism of sheep in response to an intravenous infusion of Ca

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1. The effect on calcium and phosphorus metabolism of a high rate of Ca infused directly into the blood of Ca-deficient wethers, already receiving an adequate Ca intake, has been studied by a combination of balance and radioisotope techniques.

2. The rate of Ca retention, which was already high, increased only slightly during the infusion before it reached a maximum. The surplus Ca was compensated for by a decrease in the rate of absorption and an increase in the rate of urinary Ca excretion.

3. These findings support the theory that at maximum retention the rate of Ca absorption becomes regulated according to the rate at which Ca can be stored in bone.

4. Results suggest that the decreased absorption was due to a decrease in the rate of active absorption and that the low rate remaining was due to diffusion.

5. The rate of P retention was increased by the Ca infusion, possibly as a result of the increased Ca retention.

In the sheep increased retention of calcium is brought about by a decrease in the rate of bone resorption and the rate of bone accretion remains constant (Braithwaite, 1974, 1975). In the Ca-deficient wether maximum retention of Ca occurs just as bone resorption ceases and is equal to the rate of bone accretion (Braithwaite, 1975). Since the rate of absorption of Ca from the intestine reaches a constant value at exactly the same time as retention becomes maximal, it has been suggested that Ca absorption is regulated according to the rate at which Ca can be stored in bone (Braithwaite, 1975). The maximum rate of Ca absorption possible is then equal to the rate of irreversible loss of Ca from the rapidly exchangeable pool. This regulation of Ca absorption at maximum retention appears also to occur in Ca-deficient lactating ewes (Braithwaite, 1978).

The relationship between Ca absorption, maximum Ca retention and the rate of bone accretion has now been investigated in an experiment in which a higher rate of Ca absorption than that thought to be needed for maximum retention was simulated by the infusion of calcium chloride directly into the blood of Ca-deficient sheep, now maintained on a diet adequate in Ca.

EXPERIMENTAL

Animals, housing and diet

Five 3-year-old Suffolk wethers weighing 60–70 kg were used. They were housed in metabolism cages designed for the separate collection of urine and faeces and had free access to distilled water. They were given a low-Ca basal diet of hay and concentrates (Table 1) which supplied 27·3 mg Ca/d per kg body-weight and 57·9 mg P/d per kg body-weight. Although the P intake was about adequate to supply maintenance requirements (Agricultural Research Council, 1965; National Research Council, 1968), the Ca intake was considerably less than the 55 mg/d per kg body-weight calculated from results of Braithwaite & Riazuddin, (1971) as necessary for maintenance. During the experimental period, the basal diet was supplemented with calcium carbonate so that the new Ca intake (103·3 mg/d per kg body-weight) was much greater than that needed for maintenance.

Table 1. *Composition, calcium and phosphorus content of the low-Ca basal diet* given daily to 3-year-old wether sheep*

Ingredient	Amount (g/kg body-wt)	Ca content (mg/g)	Total Ca (mg/kg body-wt)	P content (mg/g)	Total P (mg/kg body-wt)
Hay	5	4.30	21·5	2·19	10·9
Barley	5	0·56	2·8	4·40	22·0
Maize	2·5	0·03	0·1	1·56	3·9
Bran	1·25	0·16	0·2	13·02	16·3
Linseed-oil cake	0·5	3·19	1·6	9·26	4·6
Vitamin†	0·07	15·70	1·1	2·20	0·2
Whole diet			27·3		57·9

* During the experimental period $CaCO_3$ was added to the basal diet to supply an additional 76 mg Ca/kg body-wt.

† Beta Vitamin No. 3a (Cooper Nutrition Products Ltd, Witham, Essex) to supply 37·5 μg retinol equivalent and 0·775 μg cholecalciferol/kg body-wt.

Experimental design

All animals were given the low-Ca basal diet for 3 months before the start of the experiment to ensure that their skeletal stores were deficient in Ca (Braithwaite, 1974). They were then randomly divided into two groups and both groups were given the dietary supplement of $CaCO_3$. In addition, the experimental group was given an infusion of a solution of $CaCl_2$ into a jugular vein. This infusion started 3 d after the dietary supplementation and lasted for a period of 12 d.

The amount of Ca that must be absorbed by Ca-deficient wethers for maximum retention is equal to the total irreversible loss of Ca from the rapidly exchangeable pool (Braithwaite, 1975, 1978). This loss of Ca, which occurs by excretion in the urine, secretion into the intestine and accretion into bone, can be estimated from results of Braithwaite & Riazuddin (1971) at approximately 50 mg/d per kg body-weight for a 3-year-old wether. The $CaCl_2$ solution (28 mg Ca/ml) was infused at such a rate (50 mg/d per kg body-weight) that the total input of Ca into the rapidly exchangeable pool by intestinal absorption and infusion together was greater than that required for maximum retention.

Eight days after the start of the $CaCO_3$ supplementation (or 5 d after the start of the Ca infusion) Ca kinetic studies were made. A known amount (5 μCi/kg body-weight) of an aqueous solution of $^{45}CaCl_2$ (Radiochemical Centre, Amersham, Bucks) was injected into a jugular vein and samples of blood, urine and faeces were collected for a period of 1 week as previously described (Braithwaite, Glascock & Riazuddin, 1969). During this period Ca-balance measurements were made. Both groups of animals were then returned to the low-Ca basal diet for 1 month after which the treatments were reversed and the Ca kinetic studies repeated.

Methods

Kinetic analysis was done by the method of Aubert & Milhaud (1960) modified for use with sheep (Braithwaite *et al.* 1969; Braithwaite & Riazuddin, 1971). The methods used for the determination of Ca, P and radioactivity, in samples of blood, urine, faeces and food have been described previously (Braithwaite *et al.* 1969; Braithwaite, 1975).

RESULTS AND DISCUSSION

Results given in Table 2 show that when Ca was infused directly into the blood of Ca-deficient wethers, already receiving an adequate intake of Ca, the rate of Ca retention was increased by only a small amount (+12.9 mg/d per kg body-weight) compared with the

Table 2. *A comparison of the calcium and phosphorus metabolism of 3-year-old Ca-deficient wethers receiving either an adequate Ca intake or an adequate Ca intake plus an infusion of a solution of $CaCl_2$ into the jugular vein.*

(Mean values for five sheep)

	Adequate Ca intake		Standard error (residual mean square)	Significance of the difference between means
	No infusion	+Ca infusion		
Rate of ingestion of Ca (mg/d per kg body-wt)	102·5	98·5	3·1	NS
Rate of loss of Ca in faeces (mg/d per kg body-wt)	80·7	99·7	3·7	**
Rate of excretion of Ca in urine (mg/d per kg body-wt)	1·7	16·7	0.8	***
Rate of Ca retention (mg/d per kg body-wt)	+20·1	+33·0	1·8	**
Rate of secretion of Ca into intestine (faecal endogenous Ca) (mg/d per kg body-wt)	8·7	9·2	0·5	NS
Rate of absorption of Ca from intestine (mg/d per kg body-wt)	30·5	8·0	1·3	***
Ca absorbed (% Ca ingested)	29·8	8·1	1·6	***
Rate of Ca infusion (mg/d per kg body-wt)	—	50·9		
Rapidly exchangeable pool of Ca (mg/kg body-wt)	43·9	50·0	1·9	*
Slowly exchangeable pool of Ca in bone (mg/kg body-wt)	68·2	75·4	4·0	NS
Rate of accretion of Ca into bone (mg/d per kg body-wt)	36·4	36·1	1·2	NS
Rate of resorption of Ca from bone (mg/d per kg body-wt)	16·3	3·1	2·2	**
Serum Ca (mg %)	9·9	11·4	0·4	*
Rate of ingestion of P (mg/d per kg body-wt)	55·6	54·5	0·4	NS
Rate of loss of P in faeces (mg/d per kg body-wt)	46·0	30·7	2·8	**
Rate of excretion of P in urine (mg/d per kg body-wt)	2·0	2·0	0·9	NS
Apparent P absorption (P ingested – P lost in faeces) (mg/d per kg body-wt)	9·6	23·8	2·9	**
Rate of P retention (mg/d per kg body-wt)	+7·6	+21·8	2·5	**

NS, not significant. * $0{\cdot}05 > P > 0{\cdot}01$; ** $0{\cdot}01 > P > 0{\cdot}001$; *** $0{\cdot}001 > P$.

total amount of Ca infused (50·9 mg/d per kg body-weight). This result is not unexpected, however, as it has previously been shown that maximum retention of Ca occurs when bone resorption ceases (Braithwaite, 1974, 1975). Since bone resorption was already low in animals receiving the $CaCO_3$ supplement, only a slight fall was possible during the infusion before maximum retention occurred.

The remainder of the infused Ca, was compensated for by a combination of a decrease in the rate of Ca absorption (−22·5 mg/d per kg body-weight) and an increase in the rate of urinary Ca excretion (+15 mg/d per kg body-weight). A decrease in absorption has also been observed by other workers in cows made hypercalcaemic by the intravenous infusion of Ca (Ramberg & Kronfeld, 1971). Such a change in the rate of absorption of Ca adds support to the theory of Braithwaite (1975, 1978), that absorption by Ca-deficient sheep receiving an adequate Ca intake is regulated by homoeostatic mechanisms at a level just sufficient for maximum retention.

Ca absorption is now generally regarded as involving two processes, a non-saturable

diffusional one, related to intestinal Ca concentration and a saturable active one, independent of concentration but regulated according to body needs (Wasserman & Taylor, 1969; Braithwaite, 1974). Since the dietary intake of Ca remained constant in the present experiments, the decrease in absorption rate must have been due to a decrease in active transport rather than diffusion. Furthermore, since this decrease did not compensate for all the surplus Ca, the low rate of absorption remaining may not be under endocrine control and may represent that Ca absorbed by passive diffusion alone. The time (1 week) required by these Ca-deficient wethers for adaptation of the active component of Ca absorption to the increased Ca input contrasts with that (6 weeks) required by Ca-replete wethers transferred from a normal to a high Ca intake (Braithwaite, 1974). The rapid rate of response, however, may merely reflect the very large surplus of Ca input to that required for maximum retention. The mechanism involved in this adaptation was probably triggered by the resulting increase in serum Ca concentration (Table 2), which as is well known, sets in motion a train of events which involves a decreased secretion of parathyroid hormone and an increased secretion of calcitonin, a decreased secretion of 1,25-dihydroxycholecalciferol and finally a decreased rate of active absorption of Ca (Care, 1969; DeLuca, 1974, 1975; Braithwaite, 1976).

It is of interest that the rate of secretion of Ca into the intestine was not altered by the infusion, especially as there is evidence from work with non-ruminants that it is directly related to the serum Ca concentration (Gran, 1960; Toverud, 1964). Such a relationship, however, has never been confirmed in ruminants (Braithwaite, 1974; Ramberg, Mayer, Kronfeld, Phang & Berman, 1970).

Although the size of the rapidly exchangeable Ca pool and the slowly exchangeable bone pool were both increased slightly by the infusion, the increase in the bone pool was not significant. Since the rapidly exchangeable pool includes the Ca of the blood, the slight increase in its size is not unexpected.

The rates of apparent absorption and retention of P were both increased by the Ca infusion even though the P intake was unaltered. Since the rate of secretion of P into the intestine was not measured, it is not possible to decide whether the increased rate of apparent absorption was due to an increase in true absorption or to a decrease in secretion. However, since dietary P is normally absorbed in direct relation to the P intake and the excess is secreted back into the gastro-intestinal tract (Preston & Pfander, 1964; Young, Lofgreen & Luick, 1966; Young, Richards, Lofgreen & Luick, 1966), it seems likely that the increased retention was due to decreased secretion and was related to the higher rate of retention of Ca. Certainly results of previous experiments with Ca-deficient wethers suggest that Ca and P are retained in a constant ratio and that P retention is controlled according to the rate of Ca retention (Braithwaite, 1975).

Ca homoeostasis

These results illustrate the very strict control of Ca metabolism in the ruminant. Ca absorption is normally controlled according to skeletal requirements (Braithwaite, Glascock & Riazuddin, 1970; Braithwaite, 1974). When this control was circumvented by the infusion of Ca directly into the blood of the Ca-deficient sheep at such a rate that the total input into the exchangeable pool was much more than that necessary for maximum requirements, rapid adaptation took place, and homoeostasis was maintained. This adaptation involved a decrease in the rate of bone resorption to a negligible level, a decrease in the rate of active absorption of Ca, possibly also to a negligible level and finally an increase in the rate of urinary Ca excretion. The rate of accretion of Ca into bone, however, remained unaltered.

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