

Studies on the absorption and retention of calcium and phosphorus by young and mature Ca-deficient sheep

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1. A combination of a mineral balance and a radioactive technique has been used to study the effects of increased levels of dietary calcium and phosphorus on the Ca and P metabolism of young and mature wether sheep previously made Ca-deficient.
2. Ca absorption was directly related to Ca intake until an absorption maximum was reached. Young animals absorbed dietary Ca more efficiently than mature animals and had a much higher maximum rate of absorption. Apparent P absorption was directly related to P intake and maximum P absorption was also higher for the young animals.
3. Excretion of Ca in the urine and into the intestine was not altered by changes in Ca intake. Excretion of P in the urine, however, varied considerably and it is suggested that this process may be largely responsible for P homeostasis.
4. Ca retention was directly related to Ca absorption. Maximum retention was considerably higher for the young animals than for the mature animals and occurred for each group of animals at exactly the same dietary intake as maximum Ca absorption.
5. Ca and P were retained in a constant ratio of 1.25:1 and the results suggested that P retention was controlled by the rate of Ca retention.
6. Bone turnover was higher for the young than for the mature animals. Animals mobilized skeletal Ca when receiving the Ca-deficient diet and replaced their Ca losses when the dietary Ca was plentiful by changing the relative rates of bone resorption and bone accretion. Although bone resorption was mainly responsible for Ca homeostasis, bone accretion appeared to play some part in the young animals.
7. Maximum retention of Ca occurred just as the rate of bone resorption became zero and was equal to the rate of bone accretion. The lower maximum retention of Ca for mature animals was due to a lower rate of bone accretion.
8. Results suggested that when bone resorption ceased, Ca absorption was regulated by homeostatic mechanisms at a level just sufficient to maintain maximum retention, and that the maximum rates of absorption measured may not have been true maximum rates.
9. The consequences of a relationship between bone accretion and maximum Ca retention are discussed with reference to older, pregnant and lactating animals.

In calculating the dietary calcium requirements of sheep, the authors of *The Nutrient Requirements of Farm Livestock* (Agricultural Research Council, 1965) make the assumption that the efficiency of absorption of Ca decreases with age. It has recently been shown that when growth of nearly mature wethers is stimulated by treatment with oestrogen or growth hormone, the efficiency of absorption of Ca is also increased (Braithwaite, Glascock & Riazuddin, 1972; Braithwaite, 1975). This suggests that the decrease in the efficiency of absorption with increasing age may only reflect decreased Ca requirements.

Certainly young animals with high Ca requirements absorb Ca at a higher rate and with a greater efficiency than mature animals with low requirements (Braithwaite & Riazuddin, 1971). Furthermore, when requirements of mature animals are increased

Table 1. *Daily intake for dietary ingredients of the low-calcium basal diet for sheep, and the Ca and phosphorus contents of these ingredients*

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)
Straw	5	3.4	17.0	0.8	4.0
Barley	5	0.7	3.5	4.1	20.5
Flaked maize	2.5	0.03	0.1	1.5	3.7
Bran	1.25	0.4	0.5	12.7	15.9
Linseed-oil cake	0.5	3.1	2.8	8.4	4.2
Vitamin mixture*	0.07	15.7	1.1	2.2	0.2
Whole diet			25.0		48.5

* Beta Vitamin No. 3a (Cooper Nutrition Products Ltd, Witham, Essex), to supply 37.5 μ g retinol equivalent and 0.775 μ g cholecalciferol/kg body-wt.

as in pregnancy and lactation (Braithwaite, Glascock & Riazuddin, 1969, 1970) or after periods of Ca deficiency (Braithwaite, 1974) both the amount of Ca absorbed and the efficiency of absorption are increased.

The present studies were undertaken to compare young and mature animals, previously made Ca-deficient, in their ability to absorb and retain Ca.

EXPERIMENTAL

Animals, housing and diet. Eight Dorset Horn wethers were used. Four were 5-month-old growing animals weighing 25–35 kg and four were 5-year-old mature animals weighing 80–90 kg. They were housed in metabolism cages designed for the separate collection of urine and faeces, and had free access to distilled water.

It was calculated from results of Braithwaite & Riazuddin (1971) that sheep require 55 mg Ca/d per kg body-weight to supply their maintenance requirements. In the present experiments animals were given a basal diet of straw and concentrates which contained less than half this amount of Ca (Table 1). This diet was supplemented, when necessary by the addition of calcium carbonate and disodium hydrogen orthophosphate, the orthophosphate was added to ensure that Ca retention was not limited by a lack of dietary phosphorus. The low-Ca diet contained an amount of P adequate for maintenance (Agricultural Research Council, 1965) and at higher Ca intakes the dietary Ca:P ratio was kept at about 1.5.

Experimental procedure. Mature animals were given the low-Ca diet for a period of 2 months before the start of the experiment to ensure that they were deficient in Ca (Braithwaite, 1974). The young animals, because they had additional growth requirements for Ca and became deficient more quickly, were given the diet for 1 month only. Ca metabolism was studied at Ca intakes fixed at approximately 20, 50, 100, 200, 300 and 500 mg/d per kg body-weight for the mature animals and at 20, 100, 300, 400 and 500 mg/d per kg body-weight for the young animals. The corresponding P intakes were 50, 50, 70, 150, 225 and 350 mg/d per kg body-weight for the mature animals and 60, 60, 200, 300 and 365 mg/d per kg body-weight for the

young animals. Studies were done in a random manner, except those involving the low Ca intake which were done at the beginning and end of the experimental period. Each level of Ca intake was maintained for a period of 12 d and kinetic measurements, lasting 7 d, were started on the fifth day. Studies were done at monthly intervals; the animals were returned to the low-Ca basal diet for the 18 d period between studies.

Experiments using young animals were confined to the period between 6 and 11 months of age, when changes in Ca metabolism are relatively small (Braithwaite & Riazuddin, 1971). Increasing age has little effect on Ca metabolism in 5-year-old animals and it was possible to do a larger number of studies using these animals.

Methods. Ca kinetic studies were done using 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 contents and the measurement of the amount of radioactivity in samples of blood, faeces and urine have been described previously (Braithwaite *et al.* 1969). Total P content for ashed samples of food, faeces and urine was determined by the procedure of Fiske & Subbarow (1925) modified for use with an AutoAnalyzer (Technicon Instruments Corporation, Basingstoke, Hants) (Technicon Instruments Corporation, 1967).

RESULTS

The effect of increased dietary intakes of Ca and P on the Ca and P metabolism of young and mature Ca-deficient wethers are shown in Tables 2 and 3. Ca metabolism was not significantly different at the beginning and end of the experimental period for either group of animals given the Ca-deficient diet, indicating that period effects were negligible.

Absorption of Ca and P. Ca absorption was low for both groups of animals, until the dietary Ca intake exceeded 20 mg/d per kg body-weight. The rate of absorption then increased directly with intake; the efficiency of absorption was greater for the young animals than for the mature animals (Fig. 1). There was a highly significant ($P < 0.001$) linear relationship between the rate of absorption and Ca intake for young animals between intakes of 100 and 400 mg Ca/d per kg body-weight, and for mature animals between intakes of 50 and 200 mg/d per kg body-weight, and the following regression equations were calculated: young animals, $Ca_a = 8.4 + 0.2 Ca_i$; mature animals, $Ca_a = 6.0 + 0.1 Ca_i$; where Ca_a and Ca_i are Ca absorption and Ca intake (mg/d per kg body-weight) respectively.

Above dietary intakes of 200 mg/d per kg body-weight for mature animals and above 400 mg/d per kg body-weight for young animals, no significant increases in Ca absorption occurred. As a result of the greater efficiency of absorption by young animals and the higher intake reached before absorption became limited, their average maximum rate of absorption (87 mg/d per kg body-weight) was considerably greater than that for the mature animals (28 mg/d per kg body-weight).

As endogenous secretion of P into the intestine was not measured in these experiments, true rates for P absorption could not be calculated. Apparent P absorption (P intake—total P in faeces), however, was directly related ($P < 0.001$) to P intake

Table 2. *The effect on the calcium and phosphorus metabolism of young Ca-deficient wethers of increased Ca and P intakes**

	(Mean values for four animals)				Standard error (residual mean square)	Difference between mean values required for statistical significance:			
	§		P < 0.05	P < 0.01		P < 0.001			
Rate of ingestion of Ca (mg/d per kg body-wt)	24.8	98.2	274.9	402.9	505.9	24.8	12.5	18.9	30.4
Rate of loss of Ca in faeces† (mg/d per kg body-wt)	39.3	84.0	225.0	327.4	436.2	39.0	17.4	26.3	42.3
Rate of excretion of Ca in urine (mg/d per kg body-wt)	2.3	1.3	1.7	1.5	1.7	2.6	0.4	1.5	2.2
Rate of Ca retention (mg/d per kg body-wt)	-16.8	12.9	48.2	74.0	68.0	-16.8	2.3	8.1	12.2
Rate of excretion of Ca into intestine (faecal endogenous Ca) (mg/d per kg body-wt)	15.1	12.7	15.9	14.0	14.1	15.0	0.7	2.7	4.1
Rate of absorption of Ca from intestine (mg/d per kg body-wt)	0.6	26.9	65.8	89.5	83.8	0.8	2.3	7.9	11.9
Ca absorbed (% Ca ingested)	2.4	27.4	23.9	22.2	16.6	3.2	1.2	4.2	6.3
Rapidly exchangeable pool of Ca (mg/kg body-wt)	73.3	62.2	62.9	67.0	63.2	65.7	3.2	11.0	16.7
Slowly exchangeable pool of Ca in bone (mg/kg body-wt)	109.5	151.3	156.0	143.5	146.5	105.2	5.2	18.1	27.4
Rate of accretion of Ca into bone (mg/d per kg body-wt)	44.2	61.1	75.9	74.3	73.2	42.2	3.6	12.5	18.9
Rate of resorption of Ca from bone (mg/d per kg body-wt)	61.0	48.2	27.7	0.3	5.2	59.0	3.6	12.5	18.9
Rate of ingestion of P (mg/d per kg body-wt)	63.8	61.9	198.1	291.5	365.1	66.8	1.8	6.1	9.3
Rate of loss of P in faeces‡ (mg/d per kg body-wt)	59.0	45.6	142.0	194.8	259.2	59.9	5.6	19.4	29.3
Rate of excretion of P in urine (mg/d per kg body-wt)	24.7	2.5	17.0	35.9	44.7	25.5	4.7	16.4	24.9
Rate of P retention (mg/d per kg body-wt)	-19.9	13.8	39.1	60.8	61.2	-18.6	1.2	4.2	6.3
Apparent P absorption (P ingested - P lost in faeces) (mg/d per kg body-wt)	4.8	16.3	56.1	96.7	105.9	6.9	5.0	17.4	26.3
Dietary Ca:P ratio	0.39	1.59	1.39	1.38	1.39	0.37	0.07	0.25	0.37

* Each level of Ca intake was maintained for a period of 12 d and measurements were made from day 5 to day 12. There were intervals of 18 d between 12 d periods, when the animals were given the low-Ca basal diet alone. For details of diets, see Table 1; Ca and P supplements were supplied in the form of CaCO₃ and Na₂HPO₄.

† Sum of endogenous Ca and unabsorbed Ca lost/d.

‡ Sum of endogenous P and unabsorbed P lost/d.

§ Measurements made at the beginning of experiment.

|| Measurements made at the end of experiment.

Table 3. *The effect on the calcium and phosphorus metabolism of mature Ca-deficient wethers of increased Ca and P intakes**

	(Mean values for four animals)					Difference between mean values required for statistical significance:			
	§					P < 0.05	P < 0.01	P < 0.001	
Rate of ingestion of Ca (mg/d per kg body-wt)	16.2	50.7	111.2	215.2	18.3	3.8	13.0	19.7	31.6
Rate of loss of Ca in faeces (mg/d per kg body-wt)	26.1	49.4	103.0	198.8	28.2	3.3	11.3	17.1	27.4
Rate of excretion of Ca in urine (mg/d per kg body-wt)	1.0	2.0	2.1	2.0	1.3	0.8	2.7	4.1	6.6
Rate of Ca retention (mg/d per kg body-wt)	-10.9	-0.7	6.1	14.4	13.9	1.6	5.6	8.5	13.7
Rate of excretion of Ca into intestine (faecal endogenous Ca) (mg/d per kg body-wt)	10.0	9.1	9.2	9.4	10.2	1.0	3.7	5.6	8.9
Rate of absorption of Ca from intestine (mg/d per kg body-wt)	0.1	10.4	17.4	25.8	30.0	1.1	5.1	7.8	12.5
Ca absorbed (% Ca ingested)	0.6	20.5	15.6	12.0	6.0	1.2	4.2	6.3	10.1
Rapidly exchangeable pool of Ca (mg/kg body-wt)	29.3	27.8	26.7	30.1	26.3	1.6	5.6	8.5	13.7
Slowly exchangeable pool of Ca in bone (mg/kg body-wt)	27.3	31.1	30.0	31.5	28.2	2.5	8.6	13.0	20.9
Rate of accretion of Ca into bone (mg/d per kg body-wt)	14.1	12.7	12.0	15.7	13.2	1.6	5.4	8.2	13.1
Rate of resorption of Ca from bone (mg/d per kg body-wt)	25.0	13.4	5.9	1.3	-0.7	1.8	6.1	9.3	14.9
Rate of ingestion of P (mg/d per kg body-wt)	48.0	54.3	69.1	150.4	225.5	7.4	25.5	38.6	62.0
Rate of loss of P in faeces (mg/d per kg body-wt)	41.6	45.6	58.3	117.2	180.6	8.2	28.4	43.0	69.1
Rate of excretion of P in urine (mg/d per kg body-wt)	7.1	1.0	1.1	1.7	21.9	5.0	17.1	26.0	41.7
Rate of P retention (mg/d per kg body-wt)	-0.7	7.7	9.7	15.9	18.8	2.8	8.1	12.2	19.7
Apparent P absorption (P ingested - P lost in faeces) (mg/d per kg body-wt)	6.4	8.7	10.8	33.2	44.9	5.7	19.8	30.0	48.3
Dietary Ca:P ratio	0.34	0.93	1.61	1.43	1.38	0.08	0.29	0.45	0.71

* Each level of Ca intake was maintained for a period of 12 d and measurements were made from day 5 to day 12. There were intervals of 18 d between 12 d periods, when the animals were given the low-Ca basal diet alone. For details of diets, see Table 1; Ca and P were supplied in the form of CaCO₃ and Na₂HPO₄.

† Sum of endogenous P and unabsorbed P lost/d.

‡ Sum of endogenous P and unabsorbed P lost/d.

§ Measurements made at the beginning of experiment.

|| Measurements made at the end of experiment.

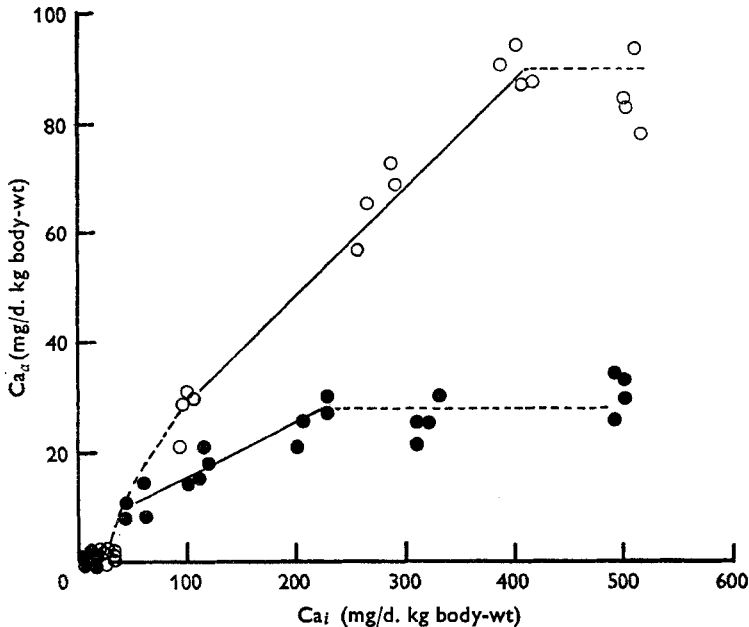


Fig. 1. Relationship between calcium intake (Ca_i) and Ca absorption (Ca_a) for wethers. (○), Young animals; $Ca_a = 8.4 + 0.2 Ca_i$. (●), Mature animals; $Ca_a = 6.0 + 0.1 Ca_i$. Animals were given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet alone. For experimental details, see Table 1 and pp. 312-3.

until absorption reached a maximum (Fig. 2) and the following regression equation, in which values were expressed as mg/d per kg body-weight, was calculated:

$$P_a = -10.9 + 0.33 P_i,$$

where P_a and P_i are the apparent P absorption and P intake respectively.

Average maximum absorption of P for young animals (101 mg/d per kg body-weight) was much higher than that for mature animals (45 mg/d per kg body-weight) and occurred at a much higher P intake. The variation in the rate of P absorption, which was considerable, particularly at the higher P intakes, appeared to be associated with variations in urinary P excretion.

Endogenous excretion of Ca and P. Excretion of Ca in the urine and into the intestine (faecal endogenous Ca) was not significantly altered in these experiments but values for faecal endogenous Ca were lower than those reported for Ca-replete animals (Braithwaite & Riazuddin, 1971). It has recently been shown, however, that a gradual decrease in faecal endogenous excretion occurs as animals become adapted to Ca-deficient diets (Braithwaite, 1974). As a result of the decreased rates of endogenous excretion, maintenance requirements for Ca (i.e. the Ca required to replace endogenous losses into urine and intestine) were lower for Ca-deficient animals than for Ca-replete animals (see Braithwaite & Riazuddin, 1971).

Excretion of P in the urine varied considerably with changes in intake. It was high

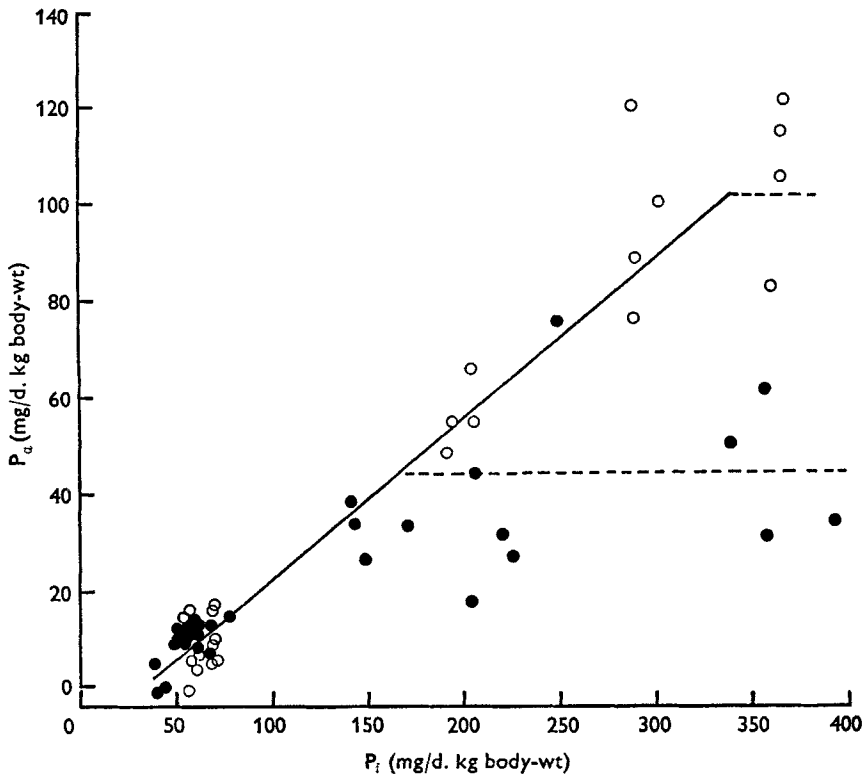


Fig. 2. Relationship between phosphorus intake (P_i) and apparent P absorption (P_a) for young (\circ) and for mature (\bullet) wethers; $P_a = -10.9 + 0.33 P_i$. Animals were given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet alone. For experimental details, see Table 1 and pp. 312-3.

for both groups of animals given the Ca-deficient diet, then decreased markedly as the Ca intake was initially increased (the P intake remaining constant) and finally increased again as the dietary levels of both Ca and P increased. The initial decrease in urinary excretion rate was not related to P intake, nor to apparent P absorption, but did appear to be inversely related to the rate of Ca retention (Fig. 3). The subsequent increase in excretion was then directly related ($P < 0.001$) to increased P absorption (Fig. 4). The following regression equations, in which values were expressed as mg/d per kg body-weight, were calculated:

$$\begin{aligned} \text{young animals, } P_u &= -20.9 + 0.63 P_a; \\ \text{mature animals, } P_u &= -8.8 + 0.76 P_a; \end{aligned}$$

where P_u and P_a are the urinary P and apparent P absorption respectively.

Retention of Ca and P. Both groups of animals lost considerable amounts of body Ca when receiving the Ca-deficient diet (Tables 2, 3). Losses of P also occurred, particularly in young animals, although the P intake was adequate. Increased intakes of Ca and P then resulted in increased retention for both elements until maximum

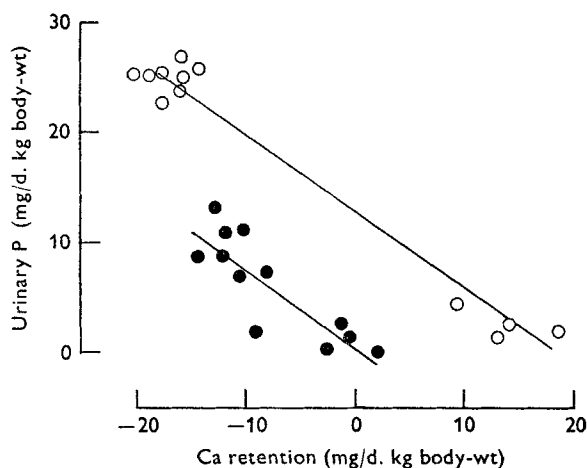


Fig. 3. Relationship between calcium retention and urinary excretion of phosphorus for young (○) and mature (●) wethers given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet alone. For experimental details, see Table 1 and pp. 312-3.

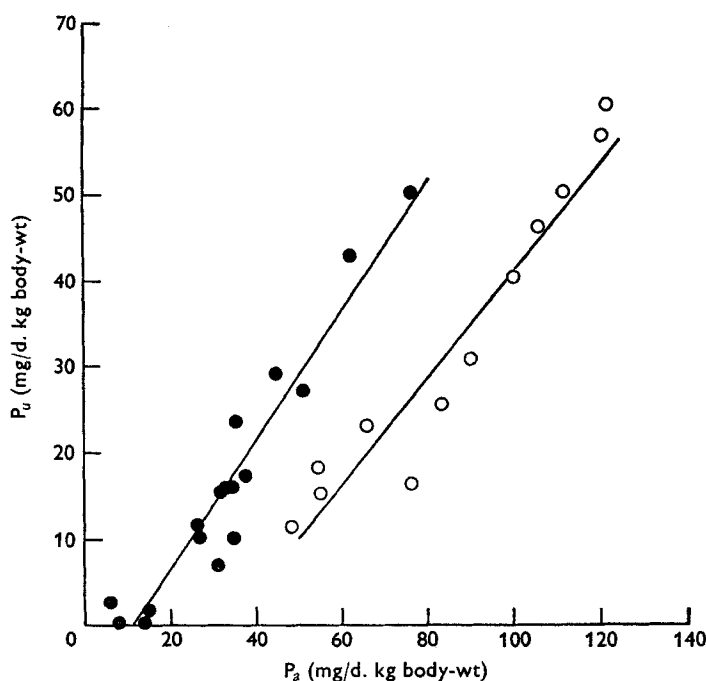


Fig. 4. Relationship between apparent absorption of phosphorus (P_a) and urinary P (P_u). (○), Young wethers; $P_u = -20.9 + 0.63 P_a$. (●), Mature wethers; $P_u = -8.8 + 0.76 P_a$. Animals were given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet alone. For experimental details, see Table 1 and pp. 312-3.

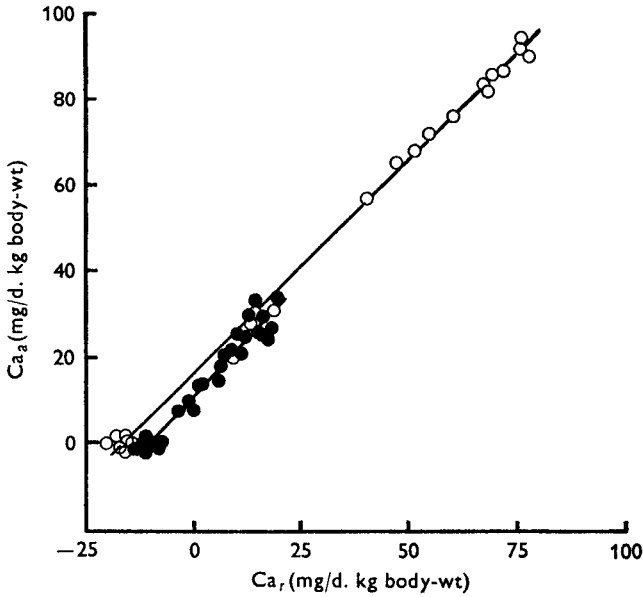


Fig. 5. Relationship between calcium retention (Ca_r) and Ca absorption (Ca_a). (○), Young wethers; $Ca_a = 16.2 + 1.0 Ca_r$. (●), Mature wethers; $Ca_a = 11.6 + 1.1 Ca_r$. Animals were given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet alone. For experimental details, see Table 1 and pp. 312-3.

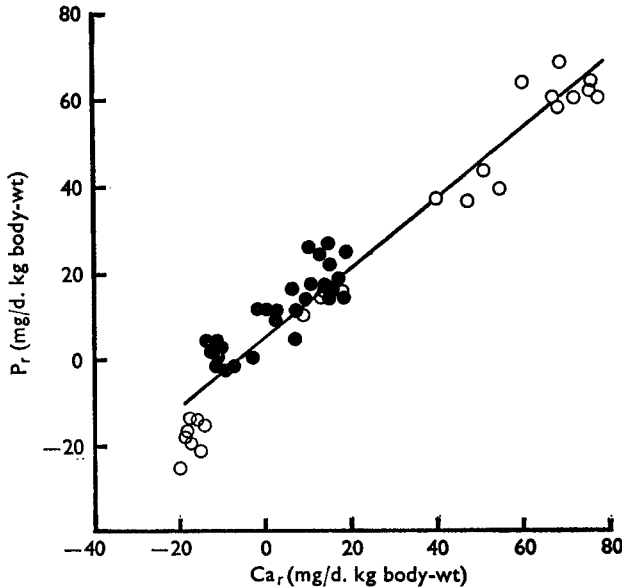


Fig. 6. Relationship between calcium retention (Ca_r) and phosphorus retention (P_r) for young (○) and mature (●) wethers; $P_r = 4.8 + 0.8 Ca_r$. Animals were given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet alone. For experimental details, see Table 1 and pp. 312-3.

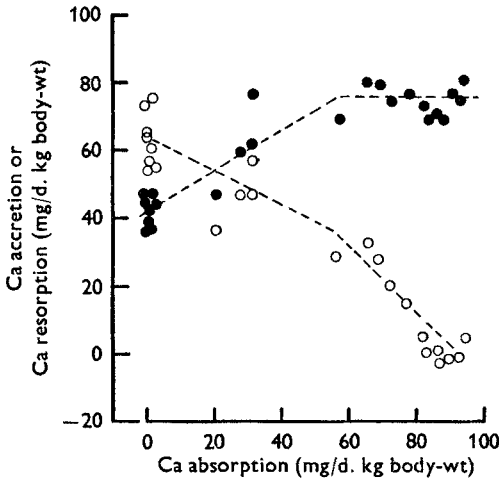


Fig. 7

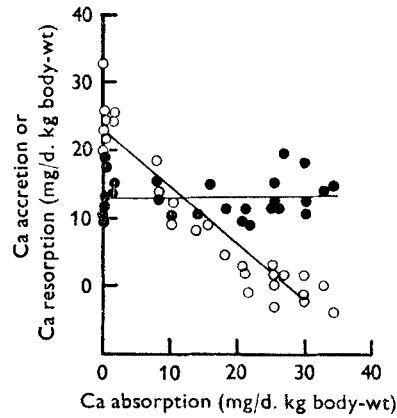


Fig. 8

Fig. 7. Relationship between accretion of calcium into bone (●), resorption of Ca from bone (○) and Ca absorption for young wethers given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet. For experimental details, see Table 1 and pp. 312-3.

Fig. 8. Relationship between accretion of calcium into bone (●), resorption of Ca from bone (○) and Ca absorption for mature wethers given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet. For experimental details, see Table 1 and pp. 312-3.

retention was reached. The maximum rate of retention of Ca for mature animals (14 mg/d per kg body-weight) was considerably lower than that for young animals (71 mg/d per kg body-weight) and occurred at a much lower Ca intake (200 mg/d per kg body-weight compared with 400 mg/d per kg body-weight for young animals). It was at exactly these same Ca intakes that maximum Ca absorption occurred.

Fig. 5 shows that there was a highly significant ($P < 0.001$) linear relationship between Ca absorption and Ca retention for young and for mature animals, and the following regression equations were calculated:

$$\text{young animals, } Ca_a = 16.2 + 1.0 Ca_r;$$

$$\text{mature animals, } Ca_a = 11.6 + 1.1 Ca_r;$$

where Ca_a and Ca_r are Ca absorption and Ca retention (mg/d per kg body-weight) respectively. These regression equations differed slightly from each other and from that ($Ca_a = 21.8 + 1.1 Ca_r$) obtained previously for sheep (Braithwaite & Riazuddin, 1971) but these differences were due to the lower maintenance requirements of the Ca-deficient animals.

Maximum P retention was also greater for the young (61 mg/d per kg body-weight) than for the mature animals (19.2 mg/d per kg body-weight), and for both groups occurred at about the same time as maximum Ca retention. Fig. 6 shows there was

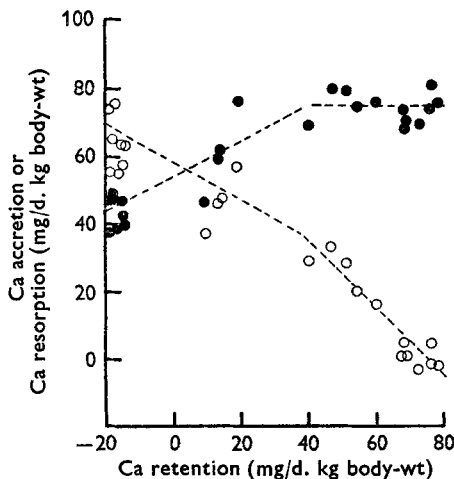


Fig. 9

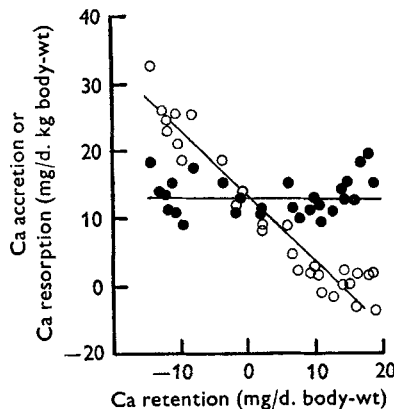


Fig. 10

Fig. 9. Relationship between accretion of calcium into bone (●), resorption of Ca from bone (○) and Ca retention for young wethers given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet. For experimental details, see Table 1 and pp. 312-3.

Fig. 10. Relationship between accretion of calcium into bone (●), resorption of Ca from bone (○) and Ca retention for mature wethers given a low-Ca basal diet supplemented with increasing amounts of Ca and P (Ca:P about 1.5 for higher Ca intakes). Each level of Ca intake was maintained for 12 d and measurements were made from day 5 to day 12, followed by 18 d on the low-Ca basal diet. For experimental details, see Table 1 and pp. 312-3.

a highly significant ($P < 0.001$) linear relationship between Ca retention and P retention. From the regression equation:

$$P_r = 4.8 + 0.8 Ca_r,$$

where P_r and Ca_r are P and Ca retention (mg/d per kg body-weight) respectively, it was calculated that the Ca:P retention ratio for these Ca-deficient animals was 1.25:1. This compares with a Ca:P ratio of 1.8 taken by the authors of *The Nutrient Requirements of Farm Livestock* (Agricultural Research Council, 1965) as corresponding to the proportion of Ca and P needed by sheep for whole body growth.

Skeletal metabolism. As previously reported (Braithwaite & Riazuddin, 1971), bone turnover was considerably lower for the mature than for the young animals.

The relationship between Ca absorption and bone turnover for young and mature animals is shown in Figs. 7 and 8. With the Ca-deficient diet Ca absorption was low and the rate of resorption of Ca from bone was higher than the rate of accretion of Ca into bone. As the Ca intake was increased, more Ca was absorbed, and the rate of bone resorption decreased until at maximum absorption it ceased altogether. Meanwhile the rate of bone accretion increased initially for the young animals but remained constant for the mature animals.

Figs. 9 and 10 show the relationship between Ca retention and bone turnover for young and for mature animals. Whereas, with the Ca-deficient diet, a higher rate of bone resorption than of bone accretion resulted in the mobilization of skeletal stores

of Ca, at high rates of Ca absorption a lower rate of bone resorption than of bone accretion enabled skeletal stores of Ca to be replenished. Maximum retention of Ca for both the young and mature animals was reached when the rate of bone resorption became zero and was approximately equal to the rate of bone accretion (see also Tables 2 and 3).

Exchangeable Ca pools. For the mature Ca-deficient animals neither the rapidly exchangeable Ca pool, nor the slowly exchangeable Ca pool for bone were significantly altered by changes in Ca intake (Table 3). For young animals the rapidly exchangeable Ca pool was also unaltered but the size of the slowly exchangeable pool for bone increased significantly as the Ca intake was initially increased (Table 2). The size of the slowly exchangeable Ca pool for bone has been found to be directly related to the rate of accretion of Ca into bone for sheep of different ages and breeds (Braithwaite & Riazuddin, 1971). Its increase in size with increased Ca intake, therefore, was probably only a consequence of the increased rate of accretion of Ca into bone.

DISCUSSION

Relationship between Ca absorption, Ca retention and bone metabolism. Although skeletal requirements for Ca must have been high for both young and mature animals, the results indicated that the young Ca-deficient animals absorbed Ca in much greater amounts and with a higher efficiency than did the Ca-deficient mature animals.

It is interesting that no absorption of Ca occurred in either group of animals at the lowest level of dietary intake. At this level of intake the Ca was present mainly as a constituent of the straw (see Table 1) in which it may be lightly bound, and the P intake was relatively high (Ca:P ratio, 1:2.5) which may also have resulted in a reduced availability of Ca. Furthermore, since Ca-binding substances have been found in the contents of the small intestine (Smith & McAllan, 1966; Smith, 1969) it seems likely that at the low Ca intake, all intestinal Ca was present in an unavailable form.

The finding that both Ca absorption and Ca retention became maximal at the same dietary Ca intake suggests that only one of these processes may have reached a true maximal rate and that the other was limited as a consequence.

Since 99% of the body Ca is present in the skeleton, total body retention of Ca can be regarded as being the same as skeletal retention, which is equal to the difference between the rate of accretion of Ca into bone and the rate of resorption of Ca from bone. In the present experiments, the rate of bone accretion, apart from an initial increase for young animals, remained remarkably constant, and at higher intakes increased retention was achieved entirely by decreased bone resorption. Maximum body retention of Ca occurred, for all animals, just as bone resorption became zero and, as would then be expected, was approximately equal to the rate of bone accretion. These findings contrast with those of Bronner (1967), who calculated Ca retention rates greatly in excess of bone accretion rates for growing rats receiving high Ca intakes. His results led him to conclude that an alternative to compartmental analysis might have to be sought. The possibility, however, that the simple two-compartment model, on which his calculations were based, was inadequate

cannot be ruled out and the present results, calculated from a more complex model, suggest that compartmental analysis does give valid results for the sheep.

The finding that maximum retention of Ca was equal to the rate of accretion of Ca into bone and coincided with the cessation of bone resorption provides strong evidence that maximum rates of Ca retention measured in these experiments were in fact true maximum rates. This leads to the suggestion that maximum rates of absorption measured may not be true maximum rates but rather that Ca absorption became regulated by homeostatic mechanisms at a level just sufficient to allow maximum retention.

The finding that maximum retention of Ca is equal to the rate of accretion of Ca into bone also explains the differences obtained for Ca metabolism of young and mature Ca-deficient animals. Many of the processes of Ca metabolism, including bone accretion, have been shown to decrease markedly in intensity with increasing age (Braithwaite & Riazuddin, 1971). Older animals, therefore, have a low maximum rate of Ca retention and, irrespective of their Ca requirements, high Ca intakes are of no benefit to them. Recently it has been shown that the administration of oestrogen or growth hormone to nearly mature wether sheep enhanced growth and reversed the normal effects of age on Ca metabolism (Braithwaite *et al.* 1972; Braithwaite, 1975). For these older animals, a stimulation of the rate of accretion of Ca into bone was accompanied by an increased ability to absorb and retain Ca.

A relationship between the rate of accretion of Ca into bone and maximum Ca retention also suggests a possible reason for the increased rates of bone accretion obtained for mature ewes during pregnancy and lactation (Braithwaite *et al.* 1969, 1970). These animals lost up to 2 g Ca/kg body-weight during late pregnancy and early lactation, when demands for Ca were high, but then replaced this lost Ca in late lactation and in the following dry period, when demands were reduced. Bone accretion rates, which in early pregnancy were about normal (8 mg/d per kg body-weight) for older animals (Braithwaite & Riazuddin, 1971) increased approximately threefold (30 mg/d per kg body-weight) during lactation. If bone accretion rates had remained at the initial low level and they do determine maximum retention, animals would have been unable to replace the Ca losses of one reproductive cycle before the onset of the next. The increase in accretion rate during lactation, however, ensured that lost reserves were replaced relatively quickly.

The initial increase in rate of bone accretion with increased Ca intake for young Ca-deficient animals but not for mature animals suggested that young animals are able to adapt to low-Ca diets by decreasing bone accretion and that this ability is lost at maturity. Certainly decreased rates of bone accretion have been reported for young Ca-deficient rats (Stauffer, Baylink, Wergedal & Rich, 1973), but similar decreases were not found for mature Ca-deficient sheep (Braithwaite, 1974).

Relationship between Ca and P metabolism. Retention of Ca was directly related to Ca absorption but P retention was more closely related to Ca retention than to apparent P absorption; the Ca:P retention ratio remained constant although there were variations in the Ca:P absorption ratio. Results indicated, therefore, that for these Ca-deficient animals P retention was controlled by the rate of Ca retention.

The initial decrease in urinary P excretion with increased Ca intake appeared to be related to changes in Ca retention, rather than to changes in P intake, which remained constant, or to apparent P absorption, which tended to increase slightly. Decreased urinary excretion of P with increased Ca intake has also been reported for rats (Clark & Rivera-Cordero, 1973), but for these animals the decrease was accompanied by a decrease in P absorption and an increase in faecal endogenous excretion of P. In the present experiments, the initial decrease in P excretion probably resulted from the decreased rate of bone resorption. With the Ca-deficient diet, the high rate of bone resorption needed to supply maintenance requirements of Ca must also have resulted in the release of large amounts of bone P. Since animals were already absorbing more than sufficient P for maintenance, the excess was presumably excreted in the urine. When the Ca intake was increased, more Ca was absorbed and bone resorption decreased. The supply of P from bone was reduced and urinary excretion of P decreased.

Ca and P homeostasis. Animals made good their maintenance requirements for Ca when receiving the Ca-deficient diet and replaced their skeletal losses when the dietary Ca was again plentiful, by altering the relative rates of bone accretion and bone resorption. Although in these experiments, as in those of Braithwaite (1974), bone resorption was the process largely responsible for Ca homeostasis, bone accretion appeared to play an important part in the young Ca-deficient animals. Furthermore, at very high Ca intakes, when the rate of bone resorption was zero and Ca retention was maximal, Ca homeostasis was probably achieved by regulation of Ca absorption.

P metabolism was closely linked to Ca metabolism in these Ca-deficient animals, and results indicated that P homeostasis was largely brought about by a control of urinary P excretion.

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