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The effect of age and level of dietary calcium intake on calcium metabolism in sheep

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1. A combination of balance and isotope techniques has been used to study the effects of age and dietary calcium content on Ca metabolism in forty-two wether sheep.

2. The amount of Ca absorbed by young growing animals varied significantly with intake. The percentage of the dietary Ca absorbed, however, remained unchanged. In older animals the amount of Ca absorbed was not altered by changes in intake, but decreased slightly with age.

3. Retention of Ca was directly related to the amount of Ca absorbed and was independent of age or breed. Furthermore, nearly all the Ca absorbed above the minimum mean amount required for maintenance was retained.

4. Faecal endogenous loss of Ca also was related to the amount of Ca absorbed. Values for faecal endogenous Ca were much lower than those used in the calculation of dietary requirements by the Agricultural Research Council (1965).

5. Urinary Ca excretion was variable, and was not related to age or changes in dietary Ca intake.

6. Increased absorption of Ca in young growing animals was accompanied by a decreased rate of bone resorption, but the rate of bone accretion remained unchanged. Changes in dietary Ca in older animals had no effect on either of these two processes. Results indicate that bone resorption is the main pathway governing Ca homoeostasis. Both the rates of Ca accretion into bone and resorption from bone decreased with age.

7. Neither the rapidly exchangeable Ca pool (P) nor the slowly exchangeable bone pool (E) was altered by changes in dietary intake in young or mature animals. Both, however, decreased in size with age.

8. The size of the slowly exchangeable pool (E) was directly related to the rate of Ca accretion into bone.

9. The results were used to calculate dietary Ca requirements of sheep gaining weight at different rates, and these values have been compared with values recommended by the Agricultural Research Council (1965).

The effect of age on calcium metabolism in ruminants in general and sheep in particular has received little attention and what few studies there are have tended to be confined to one particular aspect of metabolism. Ca requirements of sheep gaining weight at different rates have been calculated by the authors of *The Nutrient Requirements of Farm Livestock* (Agricultural Research Council, 1965). These authors emphasized, however, the lack of information available on the sheep.

The purpose of the work now presented was to study the effects of age and level of Ca intake on the various processes of Ca metabolism in the sheep and at the same time to obtain more information on its Ca requirements.

EXPERIMENTAL

Animals, housing and diet. Forty-two wethers of various breeds (Dorset Horn, Southdown and Suffolk × Half-bred) were used in these investigations. The effects of age and dietary Ca content on Ca metabolism were studied in growing (6-month-old) and in mature (16-month-old) wethers, with six animals of the same age and breed assigned to each of two diets differing in their Ca content. In addition, the effect of age was studied in 2-, 3-, 9- and 70-month-old wethers using three animals at 2 and 3 months and six at 9 and 70 months.

Table 1. *Composition and calcium content of the diet given daily to sheep* 2, 6, 9, 16 *and* 70 *months of age* (3-*month-old animals were given these dietary ingredients at* 1·5 *times these amounts*)

Ingredient	Amount (g/kg body-wt)	Ca content (mg/g)	Total Ca (mg/kg body-wt)
Hay	16	3·28–4·84	52·5–77·4
Barley	4	0·41–1·00	1·6–4·0
Flaked maize	2	0·01–0·07	0·0–0·1
Bran	1	0·72–0·74	0·7–0·7
Linseed oil cake	0·5	2·90–3·29	1·5–1·6
Mineral mixture*	0·3	184·00–187·40	55·2–56·2
Vitamin mixture†	0·028	3·78–4·41	0·1–0·1
			111·6–140·1

* Super Mindif; Boots Pure Drug Co., Nottingham. [16-month-old animals on a low Ca intake were not given the mineral mixture; 6-month-old animals on a high Ca intake were given the mineral mixture at twice this amount (i.e. 0·6 g/kg)].

† Drivite; Boots Pure Drug Co., Nottingham. To supply 125 i.u. vitamin A and 31 i.u. cholecalciferol/kg body-wt.

Animals were placed in metabolism cages designed for the separate collection of urine and faeces at least 1 month before the start of an experiment and were fed on a diet of hay and concentrates. The amount of food given was calculated according to body-weight (Table 1) but, during the several years over which these investigations have proceeded, different batches of the various dietary constituents varied considerably in Ca content, resulting in different Ca intakes (see Table 3).

In experiments on ageing, 3-month-old animals, which were in a very active state of growth, were given the dietary ingredients at 1·5 times the values shown in Table 1 and, in studies on the effect of Ca intake on Ca metabolism, dietary levels of Ca were changed by varying only the amount of mineral supplement added. Older animals (16 months of age) on the lower Ca intake were given no mineral supplement, whereas those on the higher intake were given 0·3 g/d per kg. Young animals (6 months of age), however, which are able to utilize much greater amounts of dietary Ca, were given 0·3 g/kg and 0·6 g/kg on the lower and higher intakes respectively.

The concentrate mixture was completely consumed in all experiments and the daily hay consumption was determined by collecting and weighing the residues. Animals had free access to distilled water.

Experimental procedure. A known amount (5 μCi/kg body-weight) of an aqueous solution of $^{45}CaCl_2$ (Radiochemical Centre, Amersham, Bucks) was injected into the 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.

Determination of Ca and measurement of radioactivity. Samples of blood, faeces and urine were prepared for analysis by the methods previously described (Braithwaite *et al.* 1969).

Calculation of values of the various processes of Ca metabolism. The method involves a combination of balance and radioactive techniques. The equation for the specific radioactivity–time curve of serum Ca was determined as described previously (Braithwaite *et al.* 1969). The faecal endogenous and urinary Ca losses were then calculated from the integral of the specific radioactivity–time curve and the total radioactivity excreted by each of these two pathways. The rate of Ca absorption was obtained from the equation:

$$V_a = V_i + V_f - F,$$

where V_a is the rate of absorption of Ca from the intestine, V_i the rate of ingestion of Ca, V_f the rate of excretion of Ca into the intestine (faecal endogenous Ca) and F the rate of loss of Ca in the faeces.

The method of Aubert & Milhaud (1960), as described for the sheep (Braithwaite *et al.* 1969), was then used to calculate the rates of Ca accretion into bone (V_o+) and of Ca resorption from bone (V_o-), the sizes of the rapidly exchangeable Ca pool (P) and the slowly exchangeable Ca pool (E) of bone.

Recently, the validity of these values (i.e. V_o+, V_o-, P and E) obtained by kinetic analysis has been questioned (Neuman, Terepka, Canas & Triffitt, 1968; Burkinshaw, Marshall, Oxby, Spiers, Nordin & Young, 1969). Burkinshaw *et al.* (1969) used a power series instead of using the sum of exponentials to describe the disappearance of radioactivity from the serum. They postulated a single continuously expanding exchangeable Ca pool and calculated mineralization rates which were somewhat lower than the values calculated by existing methods of kinetic analysis. We, however, have seen no advantage in changing our method of calculation and have continued to use in the present work the technique recommended by Aubert & Milhaud (1960).

RESULTS AND DISCUSSION

Ca absorption (*Table* 2). There was a highly significant difference between the amount of Ca absorbed by two groups of young growing animals of the same age (6 months) and breed fed on diets differing only in the amount of Ca present, but the percentage of dietary Ca absorbed was about the same (37–39%). These results suggest that the amount of Ca absorbed by these young animals was limited by the amount fed in the diet.

In older animals (16 months) where growth had ceased, there was no appreciable difference in the amount of Ca absorbed at the two levels of intake, resulting in a significant difference in the percentage absorption. In these animals, it was the capacity of the intestine to absorb Ca rather than the amount of Ca present in the diet that appeared to limit absorption and, unlike in young growing animals, increased absorption was not obtained simply by increasing the dietary intake. Intestinal control of absorption has also been suggested in ewes during pregnancy and lactation when insufficient Ca was absorbed to meet requirement in spite of a plentiful supply in the diet (Braithwaite *et al.* 1969; Braithwaite, Glascock & Riazuddin, 1970). Similarly, in work on cattle, Visek, Monroe, Swanson & Comar (1953) have observed a decrease in the percentage absorption with increased dietary intake. In mature sheep, it is therefore the amount of Ca absorbed that is important and possibly as in most mammals (Bronner, 1964) 'the rate of extraction of Ca from the food is a reflexion of the state of Ca metabolism and not to any significant degree of the availability of the Ca in the food'. The term 'availability' is, therefore, not very meaningful from a physiological viewpoint in these older animals.

Table 2. *Effect of level of calcium intake on Ca metabolism in young growing wethers* (6 *months of age and weighing* 30–35 *kg*), *and in mature wethers* (16 *months of age and weighing* 50–55 *kg*)

(Mean values with the standard error of the difference for six animals/group)

	Young wethers			Mature wethers		
	Low Ca intake	High Ca intake	Standard error of difference between means	Low Ca intake	High Ca intake	Standard error of difference between means
Rate of ingestion of Ca (mg/d kg body-wt)	130·1	192·6	3·7	76·4	149·3	5·9
Rate of loss of Ca in faeces* (mg/d kg body-wt)	100·0	140·1	5·0	75·5	146·8	11·1
Rate of excretion of Ca in urine (mg/d kg body-wt)	3·6	4·9	1·7	5·5	7·7	2·0
Rate of excretion of Ca into intestine (faecal endogenous Ca) (mg/d kg body-wt)	18·2	22·5	1·1	16·3	18·5	1·6
Rate of absorption of Ca from intestine (mg/d kg body-wt)	48·3	75·0	2·0	17·2	21·0	2·1
Ca absorption as % of Ca ingested	37·2	39·0	1·7	22·6	13·9	3·0
Ca balance (mg/d kg body-wt)	+26·4	+47·6	3·4	−4·6	−5·3	2·8
Rate of accretion of Ca into bone (mg/d kg body-wt)	69·2	75·6	5·0	53·1	52·6	4·2
Rate of resorption of Ca from bone (mg/d kg body-wt)	42·8	29·6	3·7	57·6	57·9	5·1
Rapidly exchangeable pool of Ca (P) (mg/kg body-wt)	63·3	69·3	5·4	62·0	63·2	2·7
Slowly exchangeable pool of Ca in bone (E) (mg/kg body-wt)	117·8	122·9	14·0	108·6	106·1	9·7

* Sum of faecal endogenous Ca and unabsorbed Ca lost/d.

Although the greatest effect of age on Ca absorption in the sheep occurred during the period of transition from a young actively growing animal to a mature adult, slight changes in absorption also occurred in the young and adult animals. In young growing animals the efficiency of absorption decreased from about 40% at 2–3 months to 36% at 9 months (Table 3). In mature animals, however, where absorption itself was the limiting factor and was independent of dietary intake, the rate of absorption decreased slightly with age (Table 3). Similar decreases in absorption with age have also been reported in cattle (Hansard, Comar & Plumlee, 1954).

Retention of Ca. In young growing animals (Table 2), increases in the rate of Ca absorption resulted in an increased retention of Ca, as shown by the increase in size of the positive balance. However, in older animals where the rate of absorption was

Table 3. *Effect of age on calcium metabolism in wether sheep*

(Mean values with their standard errors)

Age (months)	2	3	6	9	16	70
Weight (kg)	14·7± 0·5	19·0± 1·5	34·1± 0·8	37·1±1·0	53·3±1·6	92·4±3·0
Rate of ingestion of Ca (mg/d kg body-wt)	110·5± 7·9	171·6±16·1	130·1± 3·2	119·7±1·2	149·3±5·5	109·4±4·1
Rate of loss of Ca in faeces* (mg/d kg body-wt)	92·1± 6·0	123·3±11·6	100·0± 4·5	97·9±5·6	146·8±5·4	105·9±4·2
Rate of excretion of Ca in urine (mg/d kg body-wt)	3·7± 1·4	4·4± 0·6	3·6± 1·2	4·1±0·6	7·7±1·7	4·1±0·7
Rate of excretion of Ca into intestine (faecal endogenous Ca) (mg/d kg body-wt)	24·8± 2·5	24·6± 3·0	18·2± 0·9	18·1±3·7	18·7±1·5	14·1±0·5
Rate of absorption of Ca from intestine (mg/d kg body-wt)	43·2± 3·8	72·9± 8·4	43·3± 1·8	43·6±2·7	21·0±3·7	17·6±1·6
Ca absorption as % of Ca ingested	39·1± 2·1	42·4± 2·2	37·2± 1·6	36·5±2·5	13·9±2·2	16·1±1·5
Ca balance (mg/d kg body-wt)	+14·7± 3·3	+44·0± 6·0	+26·5± 2·6	+17·0±5·2	−5·3±1·7	−0·6±1·1
Rate of accretion of Ca into bone (mg/d kg body-wt)	183·2± 7·7	111·5± 2·4	69·2± 2·9	41·7±5·2	52·6±3·9	12·4±0·5
Rate of resorption of Ca from bone (mg/d kg body-wt)	168·4±10·3	67·6± 5·4	42·8± 1·6	37·0±6·5	57·9±4·2	13·1±1·1
Rapidly exchangeable pool of Ca (P) (mg/kg body-wt)	112·6± 5·3	86·8± 6·4	63·3± 3·3	59·1±5·5	63·2±1·6	32·3±1·1
Slowly exchangeable pool of Ca in bone (E) (mg/kg body-wt)	600·6±51·7	249·1±22·2	117·8±10·4	77·4±6·1	106·1±5·8	27·0±0·9
No. of animals	3	3	6	6	6	6

* Sum of faecal endogenous Ca and unabsorbed Ca lost/d.

independent of dietary Ca, there was no significant change in the state of Ca balance and there was virtually no retention. It would appear that it is only in certain circumstances that retention of Ca can be increased in mature animals. There is considerable evidence that Ca absorption in adult animals of other species can, under conditions of severe skeletal depletion, be resumed at a rate approaching that observed in infancy (Draper, 1963) and increases in absorption resulting in increased retention have also been reported in ewes in late lactation, after a period of prolonged negative balance during pregnancy and early lactation (Braithwaite *et al.* 1969, 1970).

Recent work suggests that this increase in the amount of Ca absorbed by adult animals may be a result of increased active transport of Ca across the intestine (Schachter, Dowdle & Schenker, 1960; Kimberg, Schachter & Schenker, 1961). The suggestion of Nicolaysen (1943), however, that Ca absorption is controlled by an unknown endogenous factor derived from the cellular elements of demineralized bone has not yet been verified.

Fig. 1 shows that there is a highly significant ($P < 0.001$) linear relationship ($r = 0.97$) between the amounts of Ca absorbed and retained by wether sheep, and the following regression equation was calculated:

$$V_a = 21.8 + 1.076\Delta,$$

where V_a is the amount of Ca absorbed (mg/d per kg body-weight) and Δ is the balance (mg/d per kg body-weight). This relationship appears to hold for all sheep and is independent of age, sex or breed. In fact, results for pregnant and lactating ewes (Braithwaite *et al.* 1969, 1970) also obey this equation if the Ca in the foetuses or milk is treated as though it were stored by the mother, and for comparison these values have been included in Fig. 1. From this regression equation, it can be calculated that an animal must absorb on average 22 mg Ca/d per kg body-weight in order to remain in Ca balance. If it is unable to do so, either because there is insufficient Ca available in the diet or because the ability of the intestine to absorb Ca is limiting, then the animal will move into negative balance. The value of 22 mg/d per kg is equal, therefore, to the mean amount of Ca that must be absorbed to supply the animals' maintenance requirements, i.e. to replace the endogenous losses of Ca in the urine and faeces.

From this regression equation, it can also be calculated that, for every mg Ca absorbed above the mean basal 22 mg/d per kg needed for maintenance, approximately 0.93 mg is retained by the animal.

Endogenous loss of Ca. Table 2 shows that an increased dietary intake of Ca by young growing animals resulted in a slight but significant increase in faecal endogenous loss, whereas increases in the intake of older animals had no appreciable effect. This suggests that faecal endogenous loss may be related to absorption, since the amount of Ca absorbed also increased in young animals but not in older ones. Furthermore, although the mean dietary Ca intakes of 2- and 70-month-old animals were almost identical (Table 3), the mean values of faecal endogenous Ca were quite different (24.8 and 14.1 mg/d per kg respectively) as also were the rates of absorption (43.2 and 17.6 mg/d per kg).

Fig. 2 shows that there is a significant ($P < 0.001$) relationship ($r = 0.68$) between faecal endogenous loss and the rate of Ca absorption in wether sheep.

The decrease in faecal endogenous loss with age is different from results obtained for cattle, where its value appears to remain remarkably constant throughout life (Hansard *et al.* 1954; Hansard, Crowder & Lyke, 1957).

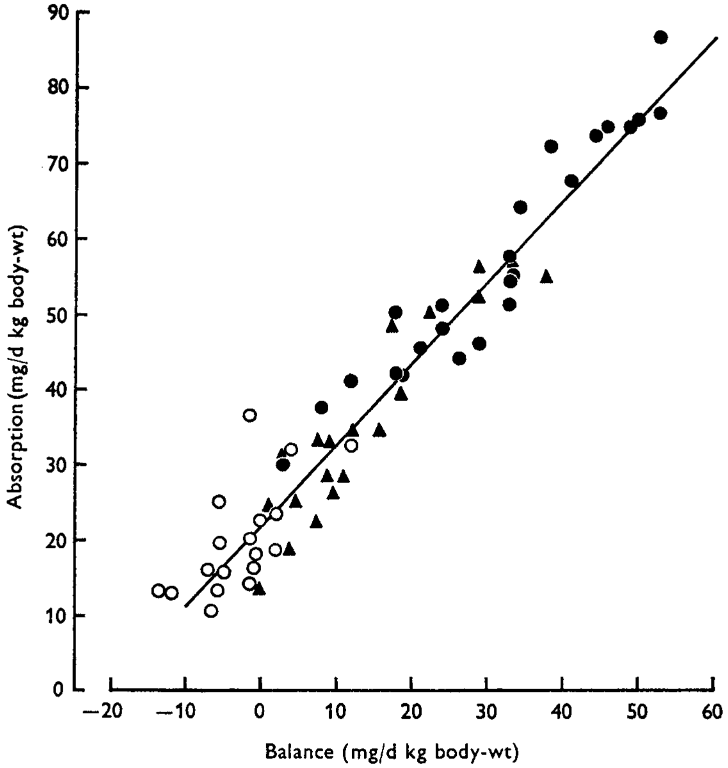

Fig. 1. Relationship between calcium balance and the rate of Ca absorption from the intestine of: ●, young growing wethers (2–9 months old); ○, mature wethers (over 9 months old); ▲, pregnant and lactating ewes. $y = 21.80 + 1.08x$. $r = 0.97$.

Furthermore, values for faecal endogenous Ca found in the present work are considerably lower than those of 35–40 mg/d per kg used by the Agricultural Research Council (1965) for the calculation of dietary requirements of growing sheep (see p. 224), but are similar to the value of 15·2 mg/d per kg obtained by them for cattle. The value of 35–40 mg was the mean of results from five groups of workers, and values varied considerably (20–60 mg/d per kg) between the groups. Recently, Field & Suttle (1969) have estimated that faecal endogenous loss in sheep is 11–22 mg/d per kg, and the present results are within this range.

Urinary excretion of Ca in sheep, although generally low, is considerably higher than in cattle. Its value was approximately 5 mg/d per kg in the present work compared with 0·8 mg/d per kg for cattle (Agricultural Research Council, 1965). In contrast with the results for faecal endogenous excretion, urinary Ca losses were not significantly altered either by changes in absorption or by age.

The total endogenous loss of Ca (i.e. in urine plus faeces) increased slightly with increased absorption, but varied considerably between animals. The mean value of 25 mg/d per kg (range 20–30) is much smaller than the value of 40 mg/d per kg used by the Agricultural Research Council (1965) for calculating dietary Ca requirements (see p. 224).

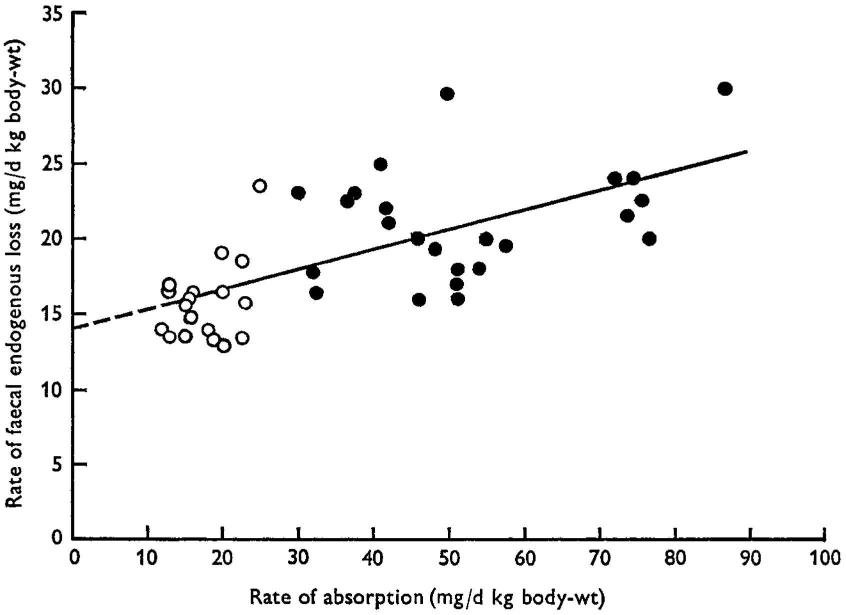

Fig. 2. Relationship between the rate of calcium absorption from the intestine and faecal endogenous loss of Ca in: ●, young growing wethers (2–9 months old); ○, mature wethers (over 9 months old). $y = 14{\cdot}11 + 0{\cdot}13x$. $r = 0{\cdot}68$.

Bone Ca metabolism. There was a significant difference in the rate of resorption of Ca from bone by the two groups of young growing animals with the different levels of Ca intake (Table 2). The rate of accretion of Ca into bone, however, altered only slightly. Ca homoeostasis in these young growing animals, therefore, appears to be controlled mainly by changes in the rate of bone resorption, as it is in lactating ewes (Braithwaite *et al.* 1969). These observations are consistent with the concept that homoeostasis is regulated by a combination of parathyroid hormone and thyrocalcitonin which control Ca metabolism by increasing or decreasing, respectively, the rate of bone resorption (Copp, 1969). They are different, however, from those obtained in man (Nordin, MacGregor & Bluhm, 1963), where changes in Ca retention in normal subjects and subjects with osteoporosis were brought about by changes in the rates of both accretion and resorption.

In mature animals, there was no significant difference at the two levels of intake in the rate of either accretion or resorption (Table 2). This, however, is not surprising, since there were no significant differences in the amounts of Ca absorbed or retained.

The rapid decrease in the rate of Ca accretion into bone in the first few months of life, followed by the slower decline later in life (Table 3), must be an effect of age, since its value was not altered by changes in dietary Ca intake. Furthermore, accretion rates have been estimated in the foetus at two stages of gestation (unpublished observations) and were found to remain fairly constant at about 400 mg/d per kg foetal body-weight, a value very much higher than that found in the 2-month-old animals in the present work.

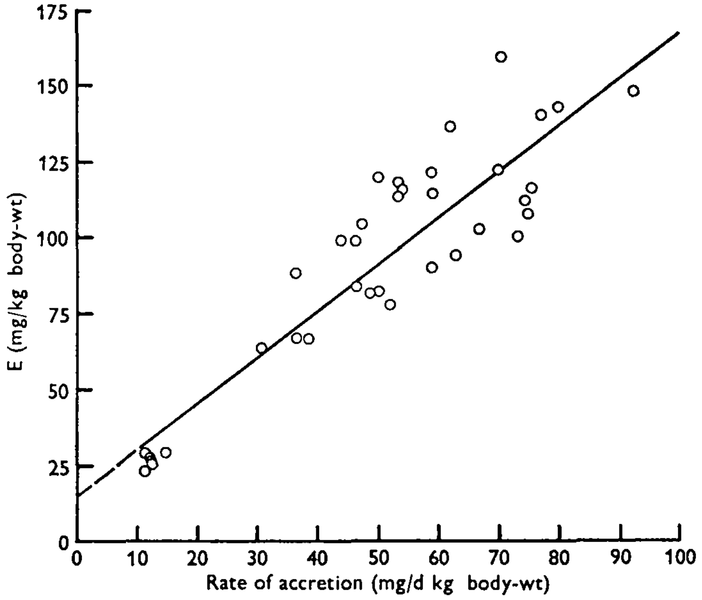

Fig. 3. Relationship between the rate of accretion of calcium into bone and the size of the slowly exchangeable Ca pool (E) of bone for sheep over 3 months of age. $y = 15{\cdot}33 + 1{\cdot}53x$. $r = 0{\cdot}91$.

Since bone resorption alters considerably in young growing animals with changes in dietary intake, the values of Table 3 are not strictly comparable, but in spite of the variations in intake, it is apparent that this process also decreased rapidly in early life and then more slowly. In mature animals, where net retention of Ca by bone was almost zero, accretion and resorption rates were very nearly equal and decreased at about the same rate with increasing age.

Exchangeable Ca pools. Table 2 shows that the rapidly exchangeable Ca pool (P) and the slowly exchangeable bone pool (E) were not significantly changed in either young growing animals or mature adults by variations in dietary intake. The changes in the sizes of these pools shown in Table 3, therefore, must result from ageing. Both P and E decreased rapidly in size in the first few months of life and then more slowly. E, however, decreased to a much greater degree than P.

Fig. 3 shows that there is a significant ($P < 0{\cdot}001$) relationship ($r = 0{\cdot}91$) in animals over 3 months of age between the size of E and the rate of Ca accretion into bone and, furthermore, preliminary experiments on the effect of hexoestrol on Ca metabolism show that treatment of wether lambs with this oestrogen results in an increase both in the rate of bone accretion and in the size of E (unpublished observations).

Calculation of the dietary Ca requirements of young sheep gaining weight at different rates. From the small amount of information available on the chemical composition of sheep carcasses, a value of 8·9 g Ca was taken by the authors of *The Nutrient Requirements of Farm Livestock* (Agricultural Research Council, 1965) as corresponding to a gain of 1 kg in body-weight, irrespective of age, weight or breed of sheep. Application of this value to results obtained in the present work gives the amounts of Ca that would be retained by animals growing at different rates. From the regression equation calculated from the results shown in Fig. 1, the amount of Ca that must be absorbed to produce a given retention can be determined. Since the percentage absorption of Ca by young growing sheep fed on a diet of hay and concentrates is independent of the Ca intake and remains constant at 35–40% (see Table 3), it is possible to use this value to calculate the dietary requirements for Ca of young sheep gaining weight at different rates.

Table 4. *Estimated calcium requirements of sheep growing at different rates*

Body-wt (kg)	Availability of Ca (%)		Ca requirement (g/d) when the sheep is gaining weight at a rate of: 50 g/d		100 g/d		200 g/d		300 g/d	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
20	40	55	2·3	2·3	3·5	3·1	5·9	4·7	8·3	6·3
30	40	50	2·8	3·3	4·0	4·2	6·4	6·0	8·8	7·7
40	35	50	3·9	4·1	5·2	5·0	8·0	6·8	10·7	8·5
50	35	40	4·5	5·4	5·8	6·4	8·6	8·4	11·3	10·4
60	35	40	5·1	6·3	6·5	7·3	9·2	9·2	—	—
70	35	40	5·7	7·2	7·1	8·2	9·6	10·1	—	—

a, present estimates; *b*, estimates of the Agricultural Research Council (1965).

The values thus obtained are shown in Table 4, together, for the purpose of comparison, with those calculated by the Agricultural Research Council (1965). On the whole, the two sets of values are in good agreement. However, the requirements of small sheep (20–30 kg body-weight) calculated from the present results are greater at the higher rates of gain than those recommended by the ARC, whereas those for larger sheep (50–70 kg body-weight) gaining at the lower rates are smaller than the corresponding values of the ARC. These differences result from different values being used by the ARC and the present authors for faecal endogenous Ca losses (40 mg/d per kg by the ARC compared with 20–30 mg/d per kg in the present work) and for efficiency of Ca absorption (40–55% by the ARC compared with 35–40% in the present work).

No requirements have been calculated for growing animals weighing less than 20 kg, because these animals would normally be on a milk diet and it is known that young milk-fed animals can absorb Ca with nearly 100% efficiency (Lengemann, Comar & Wasserman, 1957).

The present results show that Ca retention is not normally increased in mature animals by increases in dietary Ca intake. In these animals only sufficient Ca is absorbed to meet the maintenance requirements and animals tend to be in balance. It is possible, however, to calculate the minimum dietary Ca intake necessary for maintenance in these animals and at the same time it must be pointed out that Ca intakes above this minimum value are of no further benefit. The mean amount of Ca that must be absorbed for maintenance is 22 mg/d per kg (Fig. 1) and the maximum efficiency of absorption of Ca for adult sheep from a hay and concentrate diet is approximately 33%. A 50 kg sheep, therefore, requires a dietary Ca intake of 3·3 g/d for maintenance. This value compares with 4·4 g/d calculated by the Agricultural Research Council (1965) and 3·2 g/d by the National Research Council (1968).

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