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Effect of 1α-hydroxycholecalciferol on calcium and phosphorus metabolism in sheep given high or low calcium diets

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SUMMARY

The relationship between calcium and phosphorus metabolism in wether sheep given high or low Ca diets, with or without 1α-hydroxycholecalciferol (1α-OH-D_3) has been studied by a mineral balance and radioactive technique.

Ca absorption was not related to Ca intake but was stimulated by 1α-OH-D_3. More Ca was absorbed by treated animals from the high Ca diet than from the low diet and all the extra Ca absorbed was retained, increased retention being brought about largely by an increase in the rate of bone accretion.

P absorption was increased to approximately the same extent from both diets suggesting that stimulation was due to the 1α-OH-D_3 treatment rather than increased Ca absorption. Whereas the extra P absorbed from the high Ca diet was retained, together with Ca, in bone and soft tissues, that absorbed from the low Ca diet was largely excreted in the urine. It is suggested that this difference in P retention reflects a difference in availability of Ca for retention in bone and P retention was in fact found to be directly related to Ca retention.

The roles of secretion of P into the gut, absorption of P from the gut and urinary excretion of P are discussed in relation to P homoeostasis.

As absorption of P from the intestine and loss of P to bone, soft tissues and urine increased, so endogenous faecal loss decreased until it reached a value of approximately 35 mg/day per kg body weight when it remained constant. It is suggested that this value may represent the inevitable loss of endogenous P in the faeces from a hay and concentrates diet and that this minimum value may have a bearing on the calculation of P requirements.

INTRODUCTION

1α-Hydroxycholecalciferol (1α-OH-D_3) is a synthetic analogue of vitamin D which has been used to prevent post-parturient hypocalcaemia and reduce the incidence of milk fever in dairy cows (Sansom *et al.* 1976; Barlet, 1977; Gast *et al.* 1977; Sachs *et al.* 1977). Recent studies with lactating ewes and mature wethers showed that this compound markedly increased calcium and phosphorus absorption (Braithwaite, 1978, 1979*b*, 1980). An apparent relationship between these two processes led to the suggestion that increased P absorption may result merely from increased Ca absorption rather than direct stimulation by the 1α-OH-D_3 (Braithwaite, 1980). The purpose of this investigation was to clarify the relationship between P absorption, Ca absorption and 1α-OH-D_3.

MATERIALS AND METHODS

Animals, housing and diet

Twelve 3-year-old Suffolk × Scottish Blackface wethers weighing 65–75 kg were housed in metabolism cages designed for the separate collection of urine and faeces. They were maintained on a diet of hay and concentrates supplemented with vitamins and minerals (Table 1). They had free access to distilled water.

Experimental procedure

The sheep were randomly divided into four equal groups and were given either a high or a low Ca diet with or without 1α-OH-D_3 treatment according to the experimental plan shown in Table 2. P intake was kept constant. The 1α-OH-D_3 was administered intramuscularly in propylene glycol

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Table 1. *Composition of the diets given daily to wether sheep*

Ingredient	Amount (g/kg body weight)	Total Ca (mg/kg body weight)	Total P (mg/kg body weight)
Hay	5	16·7	6·3
Barley	5	7·6	15·8
Bran	5	5·2	54·8
Soya-bean meal	2·5	8·0	15·4
NaH_2PO_4	0·25	—	54·6
$CaCO_3$	0·4	160·0	—
Total	—	197·5	146·9

The diet also contained 'Rovisol oral AD_3E' (Roche Products Ltd, Dunstable, Beds) to supply (/kg body weight) 37·5 μg retinol equivalent and 0·3125 μg cholecalciferol.

For the low Ca diet, the $CaCO_3$ was omitted.

Table 2. *Experimental design*

	Period 1	Period 2
1α-OH-D_3 injected		
Group 1 (three sheep)	High Ca	Low Ca
Group 2 (three sheep)	Low Ca	High Ca
No 1α-OH-D_3		
Group 3 (three sheep)	High Ca	Low Ca
Group 4 (three sheep)	Low Ca	High Ca

Sheep were maintained on each experimental diet for 1 month before studies of calcium and phosphorus metabolism were carried out.

at a dose rate of 5 μg/day for a period of 12 days. Animals were allowed 1 month to adapt to each experimental diet before Ca and P kinetic studies were carried out, and in 1α-OH-D_3 treated sheep these studies coincided with the last 7 days of treatment.

A known amount of ^{45}Ca as calcium chloride and ^{32}P as orthophosphate (2·5 and 6 μCi/kg body weight respectively) in aqueous solution was injected into a jugular vein and samples of blood, urine and faeces were collected over a period of 7 days as previously described (Braithwaite, Glascock & Riazuddin, 1969). At the same time Ca and P balance measurements were made.

Kinetic analysis

Kinetic analysis of the Ca results was done by the method of Aubert & Milhaud (1960) modified for use with sheep (Braithwaite *et al.* 1969; Braithwaite & Riazuddin, 1971; Braithwaite & Glascock, 1976). A similar method of analysis was also used for the P results (Braithwaite, 1980). However, whereas the skeleton contains 99% of the total body Ca, it contains only 80% of the P and the remaining 20% is present in soft tissues. The rate of accretion of P into bone cannot therefore be calculated in the same way as for Ca and the equation of Aubert & Milhaud (1960) which describes the total loss of P from the exchangeable pool (V_T) must be modified to include the additional loss of P into soft tissues. Thus:

$$V_T = V_u + V_f + V_{o+} + V_{ST}$$

where V_u is the rate of excretion in the urine, V_{o+} is the rate of accretion of P into bone and V_{ST} is the rate of incorporation of P into soft tissues. Although the total loss (V_T) can be calculated in the usual way by the method of Parsons (1968) it is not possible to distinguish between V_{o+} and V_{ST} and only a combined value for these two processes can be calculated. It is recognized that this combined value may be subject to error. One problem is that calculations are based on the assumption that no radioactivity returns from the non-exchangeable pools during the period of the experiment. Whilst this is probably true of bone P it may not hold for soft-tissue P. Nevertheless previous studies (Braithwaite, 1980) show that changes in bone P metabolism resulting from 1α-OH-D_3 treatment are very similar to changes in bone Ca metabolism which suggests that this value is a useful indicator of bone metabolism.

Methods

The methods used for the measurement of the Ca content of blood, food, urine and faeces have been described previously (Braithwaite *et al.* 1969). Total P content of urine and ashed samples of food and faeces was determined by the procedure of Fiske & Subbarow (1925) modified (Technicon Instruments Corporation, 1967), for use with an autoanalyser. Serum inorganic P was measured by the same procedure after precipitation of the protein with 20% trichloroacetic acid (Manston, 1966).

Radioactivity was measured in a Packard liquid scintillation spectrometer (Model 2450B) by a dual label technique with external standardization. Samples of serum (1 ml of the trichloroacetic acid supernatant), urine (1 ml acidified with three drops of 2 M hydrochloric acid) and ashed faeces dissolved in HCl (1 ml) were counted in 10 ml of Insta-gel scintillator solution (Packard Instruments Co., Inc.).

RESULTS

Table 3 shows the mean values of the various processes of Ca and P metabolism in sheep given low or high Ca diets with or without 1α-OH-D_3 treatment and Table 4 shows the response of these processes to treatment on each of the diets. Standard errors are derived from split-plot analysis of variance in which the main plot errors (variation between sheep) are used to assess the effect of 1α-OH-D_3 injections for each diet, and the subplot

Table 3. *Calcium and phosphorus metabolism in sheep fed on diets with a high or low calcium content with or without 1α-hydroxycholecalciferol treatment*‡

	Calcium						Phosphorus					
Calcium in diet ...	High		Low				High		Low			
	Control	Treated	Control	Treated	S.E.*	S.E.†	Control	Treated	Control	Treated	S.E.*	S.E.†
Rate of ingestion	195·7	196·7	36·8	37·7	—	—	147·8	144·7	143·4	143·7	—	—
Rate of loss in faeces	193·1	157·4	42·9	28·1	3·80	3·83	143·5	113·3	127·8	105·9	11·44	5·00
Rate of excretion in urine	0·9	1·4	0·6	1·6	0·50	0·41	7·2	11·4	19·2	33·8	10·14	8·81
Rate of retention	1·7	37·9	−6·7	8·0	3·98	4·08	−2·9	20·0	−3·6	4·0	3·77	2·33
Rate of endogenous loss in faeces	11·2	14·6	11·2	12·6	1·22	1·09	50·9	38·3	44·2	40·2	5·15	5·75
Rate of endogenous secretion into gut	—	—	—	—	—	—	83·0	79·8	79·8	80·7	15·71	17·10
Rate of absorption	13·8	53·9	5·1	22·2	4·12	4·33	55·2	69·7	59·8	78·0	11·10	8·96
% absorbed	7·0	27·4	13·9	58·9	4·83	5·31	37·3	48·1	41·7	54·3	7·58	5·71
Rate of incorporation into non-exchangeable pools of bone and soft tissue	34·0	55·6	31·0	51·7	5·91	6·28	34·7	50·9	37·6	42·9	4·56	3·97
Rate of loss from non-exchangeable pools of bone and soft tissues	32·3	17·7	37·7	43·7	5·16	5·48	37·6	30·9	41·2	38·9	5·45	5·33
Serum (mmol/l)	2·56	3·28	2·30	2·63	0·150	0·143	2·69	4·01	2·95	3·20	0·313	0·214

* Standard error of a difference between means for control periods on different diets, or between treated animals on different diets (10 D.F.).

† Standard error of a difference between means for animals on the same diet in the control period and when treated with 1α-hydroxycholecalciferol (10 D.F.).

‡ Results, in mg/day per kg body weight, are means of six animals per group.

Table 4. *The response of various processes of calcium and phosphorus metabolism in sheep, fed on diets with a high or low calcium content, to the injection of 1α-hydroxycholecalciferol*†

	Calcium			Phosphorus		
Calcium in diet	High	Low	Difference	High	Low	Difference
Rate of ingestion	1·0	0·9	0·1	−3·1	0·3	−3·4
Rate of loss in faeces	−35·7***	−14·8**	−20·9***	−30·2*	−21·9 NS	−8·3 NS
Rate of excretion in urine	0·5 NS	1·0 NS	−0·5 NS	4·2 NS	14·6 NS	−10·4 NS
Rate of retention	36·2***	14·7**	21·5***	22·9***	7·6 NS	15·3**
Rate of endogenous loss in faeces	3·4*	1·4 NS	2·0 NS	−12·6*	−4·0 NS	−8·6 NS
Rate of endogenous secretion into gut				−3·2 NS	0·9 NS	4·1 NS
Rate of absorption	40·1***	17·1**	23·0***	14·5 NS	18·2 NS	−3·7 NS
% absorbed	20·4***	45·0***	−24·6***	10·8 NS	12·6 NS	−1·8 NS
Rate of incorporation into non-exchangeable pools of bone and soft tissues	21·6**	20·7**	0·9 NS	16·2**	5·3 NS	10·9*
Rate of loss from non-exchangeable pools of bone and soft tissues	−14·6*	6·0 NS	−20·6**	−6·7 NS	−2·3 NS	−4·4 NS
Serum (mmol/l)	0·72***	0·33 NS	0·39*	1·32**	0·25 NS	1·07**

* $0{\cdot}05 > P > 0{\cdot}01$; ** $0{\cdot}01 > P > 0{\cdot}001$; *** $0{\cdot}001 > P$; NS, $P > 0{\cdot}05$.

† Values in mg/day per kg body weight are means of six animals.

errors (sheep and diet interaction) are used to assess the diet differences in the injected and control groups.

Calcium metabolism

Control animals failed to absorb sufficient Ca from the low Ca diet to meet their maintenance requirements (i.e. the inevitable losses of Ca in urine and digestive juices) and Ca retention was negative. The rate of absorption was slightly higher from the high Ca diet but was still only just enough for maintenance.

Treatment with 1α-OH-D_3 resulted in a marked increase in the rate of absorption of Ca from both diets, the increase from the high Ca diet being much greater than from the low Ca diet. Absorption from the low Ca diet, however, was much more efficient.

Since urinary excretion of Ca was not significantly altered by treatment, and since faecal endogenous loss was increased slightly only on the high Ca diet, virtually all the extra Ca absorbed from both low and high Ca diets was retained. Because of the greater absorption rate, retention from the high Ca diet was significantly greater than from the low Ca diet. Figure 1 shows that there was a highly significant ($P < 0{\cdot}001$) linear relationship ($r = 0{\cdot}99$) between Ca absorption (Ca_a) and Ca retention (Ca_r) and the regression equation

$$Ca_a = 14{\cdot}0 + 1{\cdot}07\ Ca_r,$$

in which values are expressed in mg/day per kg body weight, agrees well with that ($Ca_a = 19{\cdot}7 +$ 1·01 Ca_r) reported previously for sheep of various age, sex and breed (Braithwaite & Riazuddin, 1971).

On both diets, increased bone accretion was largely responsible for the increased retention by treated animals, but on the high Ca diet there was also a reduction in the rate of bone resorption.

Phosphorus metabolism

The rate of absorption of P was high in control animals and was unaltered by Ca intake. Since urinary excretion and faecal endogenous loss of P were also high, retention was slightly negative. Absorption of P was increased by 1α-OH-D_3 treatment from both high and low Ca diets but possibly because of the considerable variability, the increases were not significant. The amount of P in the faeces was reduced by treatment, as also was the endogenous faecal loss. These reductions were significant, however, only on the high Ca diet.

Although P absorption was increased by treatment to approximately the same extent from both diets, P retention was increased markedly on the high Ca diet not substantially changed on the low Ca diet. This difference in P retention was accounted for by a greater rate of urinary excretion of P on the low Ca diet but because of the large variability, this increase in excretion was not significant.

P retention was not related to P absorption but

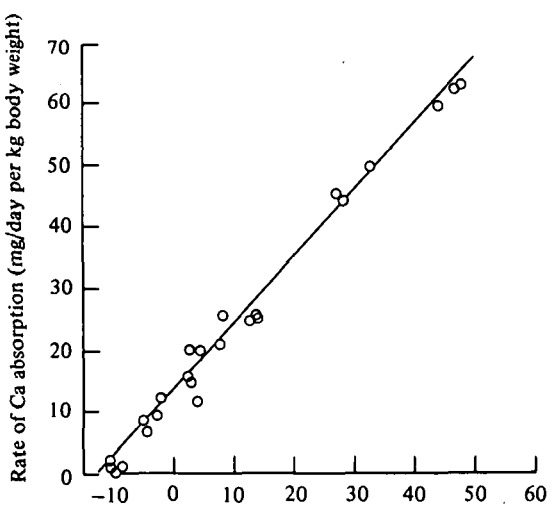

Fig. 1. Relationship between the rate of Ca retention and the rate of Ca absorption by wether sheep given high or low Ca diets with or without 1α-hydroxycholecalciferol treatment. $y = 14{\cdot}0 + 1{\cdot}07x$; $r = 0{\cdot}99$.

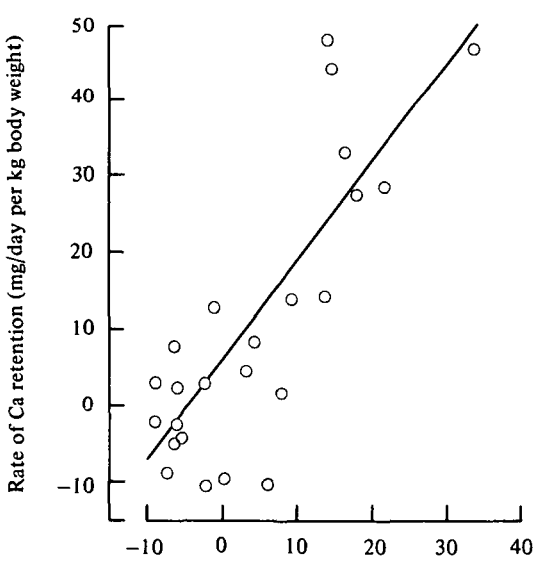

Fig. 2. Relationship between the rate of P retention and the rate of Ca retention by wether sheep given high or low Ca diets with or without 1α-hydroxycholecalciferol treatment. $y = 5{\cdot}8 + 1{\cdot}3x$; $r = 0{\cdot}81$.

was related to Ca retention. Figure 2 shows there was a highly significant ($P < 0.001$) linear relationship ($r = 0.81$) between Ca retention (Ca_r) and P retention (P_r) described by the following regression equation

$$Ca_r = 5.8 + 1.3\ P_r$$

in which values are expressed in mg/day per kg body weight.

Although the increased retention of P by treated animals given the high Ca diet was achieved mainly by an increased rate of accretion of P into the non-exchangeable pools of bone and soft tissues, there was also a small, but non-significant, decrease in the loss of P from these pools.

DISCUSSION

Results show that Ca absorption was not related to Ca intake but was stimulated by 1α-OH-D_3. Furthermore, they suggest that absorption from the high Ca diet was probably limited by the capacity of the intestine to absorb Ca but from the low diet was limited by the availability of dietary Ca, which for this particular hay and concentrates diet was approximately 59% of the total Ca intake.

It is surprising that control animals were unable to absorb enough Ca from the low Ca diet to meet their maintenance requirements, expecially in view of the fact that the efficiency of absorption was much lower than the 59% observed in treated animals. However, previous results suggest that a considerable loss of skeletal reserves of Ca has to occur before animals can adapt to a low Ca intake (Braithwaite, Glascock & Riazuddin, 1970; Braithwaite & Glascock, 1976). In the present study losses of skeletal reserves may have been insufficient for a stimulation of absorption to have occurred.

That absorption from the high Ca diet was regulated in control animals at a level just sufficient to replace maintenance losses, agrees well with previous findings (Braithwaite, 1974). This regulation is thought to be achieved by parathyroid hormone mediated control of the hydroxylation of 25-hydroxycholecalciferol to 1-α-25-hydroxycholecalciferol (1α,25$(OH)_2D_3$), the metabolite of vitamin D now generally considered to be responsible for the stimulation of Ca absorption (Braithwaite, 1976). When this hydroxylation step was by-passed by treatment with 1α-OH-D_3, which, irrespective of the parathyroid hormone status, is rapidly converted to 1α,25$(OH)_2D_3$ (Holick *et al.* 1976), absorption of Ca was stimulated to a level much greater than that required to replace maintenance losses. The extra Ca absorbed, although surplus to requirements, was not, as might have been expected, immediately excreted, but was retained, thus providing further evidence for the suggestion (Braithwaite, 1979*a*) that sheep may lack a mechanism for getting rid of unwanted Ca.

Studies on various species have now shown that P absorption is increased by vitamin D and its metabolites (Harrison & Harrison, 1961; Wasserman & Taylor, 1973; Chen *et al.* 1974; Fox & Care, 1976; Braithwaite, 1978). There is controversy, however, over the actual mechanism by which this increase occurs (Wasserman, 1975; Norman, 1978). Previous studies with sheep (Braithwaite, 1980) suggested that the increased absorption of P associated with 1α-OH-D_3 treatment may be a result of increased Ca absorption rather than a direct effect of 1α-OH-D_3. Results now show that this is not so. Whereas the 1α-OH-D_3 mediated increase in Ca absorption was markedly different on the high and low Ca diets, the increase in P absorption was approximately the same. These results suggest rather that P absorption is stimulated directly by 1α,25$(OH)_2D_3$.

Whereas on the high Ca diet, virtually all the extra P absorbed by treated animals was retained, on the low Ca diet only about half was retained and the other half was excreted in the urine. This difference in P retention may be explained on the basis of changes in Ca metabolism. Ca absorbed in excess of maintenance requirements is normally retained in bone (Braithwaite, 1979*a*, 1980), the inorganic material of which is mainly hydroxyapatite. Increased Ca retention therefore is normally accompanied by increased P retention. Ca was absorbed from the high Ca diet, during treatment, at a rate much greater than that required for maintenance. The surplus Ca was then retained, together with P, in the bone, the increased retention being achieved by a decrease in the rate of bone resorption relative to that of bone accretion. In contrast, Ca was absorbed from the low Ca diet during treatment at a rate only slightly greater than that required for maintenance. Because of the lack of available Ca, the extra P absorbed could not be retained in bone and was instead eliminated in the urine. P retention, therefore, appeared to be controlled according to Ca retention, and as shown in the results section, there was a highly significant linear relationship between these two processes.

Since the skeleton contains only 80% of the total body P and the remaining 20% is present in soft tissues, the P retained during treatment was probably not all retained in bone. Unfortunately, however, it is not possible to distinguish between accretion of P into bone and incorporation into soft tissues by the techniques used in this study.

Many workers have now shown that dietary P is absorbed in direct relation to P intake and that the P absorbed in excess of requirements is lost in the faeces (Lueker & Lofgreen, 1961; Preston & Pfander, 1964; Young, Lofgreen & Luick, 1966;

Young, Richards, Lofgreen & Luick, 1966). Since urinary excretion of P is normally very low, P homoeostasis is generally thought to be achieved by control of endogenous secretion into the gut, which occurs mainly in the saliva. It is therefore somewhat surprising to find that the surplus P, absorbed from the low P diet by treated animals, was lost in the urine rather than in the intestine and that in fact faecal endogenous loss of P was decreased slightly rather than increased. When the rates of total secretion of P into the upper gut are calculated by the method used by Young, Lofgreen & Luick (1966), which is based on the assumption that the endogenous P of saliva is absorbed with the same efficiency as dietary P, it is found that values are fairly constant and unrelated to P intake, 1α-OH-D_3 treatment or even serum P concentration. In this respect, the results differ from those of previous studies (Braithwaite, 1980) in which total endogenous secretion of P appeared to be stimulated by 1α-OH-D_3 treatment and to be directly related to the serum P concentration. The reason for this difference is at present unknown.

Since the rate of loss of endogenous P in the faeces decreased as the efficiency of absorption of dietary P increased whilst the rate of total secretion of P into the upper gut remained unchanged (Table 3), P homoeostasis may be brought about by control of the rate of P absorption rather than by control of the rate of endogenous secretion. This conclusion was also reached by Towns, Boston & Leaver (1978) from theoretical considerations and by Young, Lofgreen & Luick (1966) from P kinetic studies. The present studies, which show that P absorption is stimulated by 1α-OH-D_3, suggest that this control of absorption may be mediated by 1α,25$(OH)_2D_3$.

In certain circumstances, urinary excretion of P may also have a role in P homoeostasis. In treated animals given the low Ca diet, absorption of P was fixed at a high level because normal homoeostatic control was overcome by the 1α-OH-D_3. The only means of eliminating surplus P was then by increased excretion in the urine. Increased urinary excretion of P has also been found to occur when endogenous loss of P in the saliva was prevented by ligation of the parotid ducts (Tomas & Somers, 1974).

The possibility that high rates of urinary excretion are not always related to P homoeostasis but may sometimes occur as an obligatory loss in response to some as yet unidentified factor cannot be entirely ruled out. Certainly high rates of urinary excretion do occasionally occur for no apparent reason and are usually associated with high rates of P absorption (Manston & Vagg, 1970; Meyer, 1972; G. D. Braithwaite, unpublished observations). It is possible therefore that a high rate of P absorption may be the response to, rather than the cause of, a high rate of urinary excretion.

As the efficiency of absorption of P and the losses of P to bone and urine were increased, so endogenous faecal loss tended to decrease until it reached a value of approximately 35 mg/day per kg body weight when it remained relatively constant (see Fig. 3). This minimum value probably represents the inevitable loss of endogenous P in the faeces

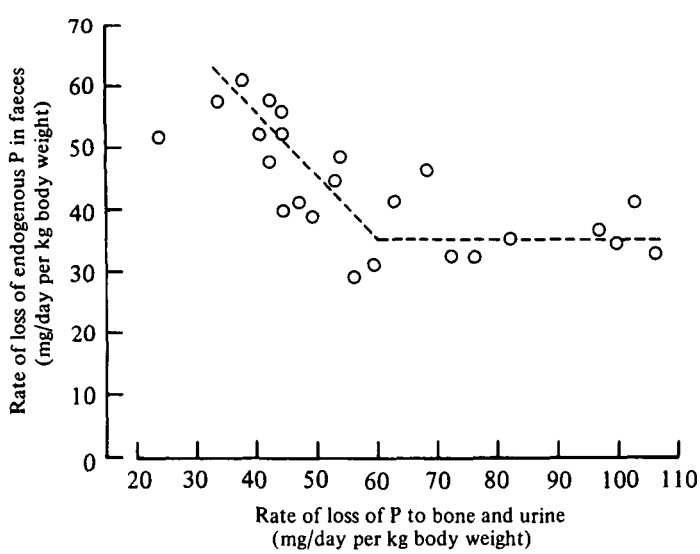

Fig. 3. Relationship between the rate of loss of P to bone and urine and the rate of loss of endogenous P in the faeces of wether sheep given high or low Ca diets with or without 1α-hydroxycholecalciferol.

from the hay and concentrates diet and it may have some bearing on calculations of P requirements (Agricultural Research Council, 1965; National Research Council, 1968) which depend upon a value for the minimum rate of endogenous loss. Recently very low rates of endogenous loss have been reported in animals given P deficient semi-purified diets (Sykes & Dingwall, 1976). These findings have led to the suggestion that the values used previously by the Agricultural Research Council and National Research Council to calculate requirements were too high. The present results suggest that the minimum value for endogenous secretion in animals given a hay and concentrates diet containing adequate P is not very different from that already used to calculate requirements (Agricultural Research Council, 1965; National Research Council, 1968).

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