

The effect of 1- α -hydroxycholecalciferol on calcium and phosphorus metabolism in the lactating ewe

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1. The effect of 1- α -hydroxycholecalciferol (1 α -OH-D₃) on calcium and phosphorus metabolism has been studied in ewes at peak lactation by a combination of a mineral balance and a radioisotope technique.
2. The rate of Ca absorption was substantially higher in the treated ewes than in controls. The rates of endogenous loss of Ca into urine, faeces and milk, however, were only slightly higher.
3. In consequence, the net retention of Ca was increased and the loss of skeletal reserves of Ca normally associated with peak lactation, prevented.
4. Although the rate of bone accretion increased slightly, the increase in skeletal retention of Ca resulted mainly from a decrease in the rate of bone resorption.
5. This finding conflicts with the generally held belief that bone resorption is increased by cholecalciferol treatment.
6. The rates of apparent absorption and retention of P were increased by the treatment probably as a result of a direct effect of the 1 α -OH-D₃ on P absorption.
7. These results provide a possible explanation of the beneficial effect of 1 α -OH-D₃ in preventing parturient paresis (milk fever) in the dairy cow.

Cholecalciferol has been used for many years with varying success in the prevention of parturient paresis (Hibbs & Pounden, 1955; Hibbs & Conrad, 1960), the calcium deficiency disorder of dairy cows which occurs at the onset of lactation when demands for Ca and phosphorus are substantially increased (Symonds, Manston, Payne & Sanson, 1966). It is generally assumed that the effectiveness of cholecalciferol in preventing this disorder is due to its property of increasing the blood Ca concentration by increasing its absorption from the intestine and its mobilization from bone (Carlsson, Lindquist & Magnusson, 1952; Fraser & Kodicek, 1973). The use of cholecalciferol, however, has several drawbacks. It is only slow-acting and to be effective must be administered several days before the cow calves (Hibbs & Conrad, 1960). Furthermore, the massive doses required may be toxic (Cole, Chamberlain, Hibbs, Pounden & Smith, 1957; Manston & Payne, 1964).

Recently it has been shown that cholecalciferol is hydroxylated in the liver to 25-hydroxycholecalciferol (25-OH-D₃) and subsequently in the kidney to 1 α ,25-dihydroxycholecalciferol (1 α ,25(OH)₂D₃) which is generally believed to be the active metabolite (DeLuca, 1975; Wasserman, 1975). These metabolites of cholecalciferol might afford better protection against parturient paresis than cholecalciferol itself. Unfortunately, 1 α ,25(OH)₂D₃ is not readily available and 25-OH-D₃, although used with some success in the prevention of parturient paresis (Olsen, Jorgensen, Schultz & DeLuca, 1973), has the disadvantage that its conversion to 1 α ,25(OH)₂D₃ is strictly regulated by a number of factors, one of which is plasma Ca concentration (Fraser & Kodicek, 1973).

1- α -Hydroxycholecalciferol (1 α -OH-D₃) is a synthetic analogue of cholecalciferol which is rapidly hydroxylated to 1 α ,25(OH)₂D₃ (Holick, Taleva, Holick, Schnoes, DeLuca & Gallagher, 1976). This compound has already been shown to prevent post-parturient hypocalcaemia and to reduce the incidence of parturient paresis in the dairy cow (Sansom, Allen,

Table 1. *Daily intake of dietary ingredients by ewes in early lactation*

Ingredient	Intake (g/kg body-wt)	Total calcium (mg/kg body-wt)	Total phosphorus (mg/kg body-wt)
Hay	20	90.6	14.2
Barley	10	4.2	36.0
Bran	2	1.3	21.7
Soya-bean meal	3	7.0	21.0
Mineral mixture*	0.2	49.0	13.6
Vitamin mixture†	0.07	1.1	0.2
Dicalcium phosphate	0.75	172.8	136.1
Total		326.0	242.8

* Sheep mineral supplement (ICI Ltd, Pharmaceuticals Division, Macclesfield, Cheshire).

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

Davies, Hoare, Stenton & Vagg, 1976) but its effect on the various processes of Ca and P metabolism have not been investigated in ruminants.

The sheep, like the cow, is normally unable to absorb enough dietary Ca to meet the high demands of peak lactation and as a result, body reserves of Ca are mobilized (Braithwaite, Glascock & Riazuddin, 1969, 1970). The present studies were done to investigate the possibility of preventing this loss of body Ca reserves at peak lactation by treatment with 1α -OH- D_3 . At the same time, it was hoped to identify the processes of Ca and P metabolism controlled by the 1α -OH- D_3 and so elucidate the probable mechanism of action of this compound in reducing post-parturient hypocalcaemia and parturient paresis in the dairy cow.

EXPERIMENTAL

Animals, housing and diet. Ten 3-year-old Masham ewes weighing 55–60 kg were used. At 2 months after mating they were placed in individual metabolism cages designed for the separate collection of urine and faeces and were given a diet of hay and concentrates, the composition of which was adjusted at monthly intervals to meet the changing nutritional requirements of pregnancy and lactation (Agricultural Research Council, 1965). The composition of the diet given in lactation is shown in Table 1. The lambs were removed 2 d after birth and the ewes were then machine-milked twice daily (Treacher, 1970). Half the daily concentrate ratio was given at each milking.

Experimental procedure. At 1 week after lambing the ewes were randomly divided into two groups. One group was given daily for 10 d an intramuscular injection of 1α -OH- D_3 (5 μ g/d in 0.2 ml propylene glycol). The other group was untreated and acted as a control.

Ca kinetic studies were carried out 3 d after the start of the 1α -OH- D_3 treatment. A known amount (5 μ Ci/kg body-weight) of $^{45}\text{CaCl}_2$ (Radiochemical Centre, Amersham, Bucks) in aqueous solution was injected into a jugular vein immediately after the morning milking and samples of blood, urine, faeces and milk were collected for a period of 1 week. During this period Ca and P balance measurements were made.

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 the Ca content and measurement of radioactivity in samples of blood, urine, faeces and milk have been described previously (Braithwaite *et al.* 1969). Total P content of ashed samples of food, urine, faeces and milk was determined by the procedure

Table 2. *The effect of 1- α -hydroxycholecalciferol (1 α -OH-D₃ administration on the calcium and phosphorus metabolism of ewes in early lactation*(Mean values with their standard errors for five animals/group; result of tests of significance as determined by the Students *t* test)

	Control ewes		1 α -OH-D ₃ -treated ewes		Statistical significance of difference between means
	Mean	SE	Mean	SE	
Rate of ingestion of Ca (mg/d per kg body-wt)	315.7	4.3	315.9	6.5	NS
Rate of loss of Ca in faeces (mg/d per kg body-wt)	296.1	6.7	253.7	11.0	*
Rate of excretion of Ca in urine (mg/d per kg body-wt)	3.5	1.4	13.1	1.1	***
Rate of secretion of Ca into milk (mg/d per kg body-wt)	42.2	5.6	49.2	7.6	NS
Rate of Ca retention (mg/d per kg body-wt)	-26.1	9.4	-0.1	11.9	*
Rate of secretion of Ca into intestine (faecal endogenous Ca) (mg/d per kg body-wt)	21.5	2.7	24.4	1.7	NS
Rate of absorption of Ca from intestine (mg/d per kg body-wt)	41.1	2.8	86.6	6.9	***
Ca absorbed (% Ca ingested)	13.0	0.9	27.5	2.2	***
Rapidly exchangeable pool of Ca (mg/kg body-wt)	47.8	4.4	52.5	3.7	NS
Slowly exchangeable pool of Ca in bone (mg/kg body-wt)	31.9	4.3	35.5	6.4	NS
Rate of accretion of Ca into bone (mg/d per kg body-wt)	16.8	3.1	23.6	1.5	•
Rate of resorption of Ca from bone (mg/d per kg body-wt)	42.9	9.7	23.7	17.4	***
Rate of ingestion of P (mg/d per kg body-wt)	237.3	2.9	237.6	5.0	NS
Rate of loss of P in faeces (mg/d per kg body-wt)	215.8	3.5	180.2	12.6	*
Rate of excretion of P in urine (mg/d per kg body-wt)	6.6	2.0	16.5	8.9	NS
Rate of secretion of P into milk (mg/d per kg body-wt)	31.4	2.9	30.8	4.2	NS
Rate of retention of P (mg/d per kg body-wt)	-16.5	3.4	+10.1	5.4	**
Apparent absorption of P (P ingested - P lost in faeces) (mg/d per kg body-wt)	21.5	3.3	57.4	13.8	*
Serum Ca (mmol/l)	2.32	1.12	2.9	0.08	**
Serum P (mmol/l)	2.1	0.09	2.72	0.13	**

NS, not significant. * 0.05 > *P* > 0.01, ** 0.01 > *P* > 0.001, ***0.001 > *P*.

of Fiske & Subbarow (1925) modified (Technicon Instruments Corporation, 1967) for use with an AutoAnalyzer.

RESULTS

Results of these studies are summarized in Table 2 and show considerable differences between the treated and untreated ewes in both their Ca and P metabolism.

The major effect of the 1 α -OH-D₃ treatment was to increase the mean rate of intestinal absorption of Ca from 41.1 mg/d per kg body-weight in the control group to 86.6 mg/d per

kg body-weight in the experimental group. Since the Ca intake was the same in both groups, this increase occurred as a result of an increased efficiency of absorption of the dietary Ca.

The rate of loss of Ca in the urine was significantly increased by the treatment. Losses into the milk and intestine were also slightly higher but not significantly so. Since there was a greater increase in the rate of absorption of Ca than in the rate of loss (into urine, milk and intestine), the net retention of Ca was increased. Whereas the control animals did not absorb enough dietary Ca to meet all their immediate needs and retention was negative, the treated animals did, and were approximately in Ca balance.

To make good the deficit between Ca absorbed and total Ca lost, control animals had to draw on skeletal reserves of Ca and the rate of bone resorption was high relative to that of bone accretion. Treatment of the experimental group with 1α -OH- D_3 , however, resulted in a large decrease in the rate of bone resorption and a small increase in the rate of bone accretion until the two processes were almost in equilibrium.

The higher rates of absorption and retention of Ca in the treated ewes resulted in a significant increase in their serum Ca concentration. In spite of this increase, however, no changes were observed in the size of either the rapidly exchangeable Ca pool, of which the serum Ca forms a part, or the slowly exchangeable bone pool.

Although not all the major processes of P metabolism were measured, the 1α -OH- D_3 treatment seemed to have a similar effect on P metabolism as on Ca metabolism. Thus the apparent absorption of P (ingested P – faecal P) showed a significant increase and so did the serum P concentration and the net P retention. Loss of P in the urine was also increased slightly but loss in the milk was unchanged.

DISCUSSION

These results show that the rate of absorption of dietary Ca by lactating ewes was increased to such an extent during treatment with 1α -OH- D_3 that the negative retention usually associated with peak lactation (Braithwaite *et al.* 1969, 1970; Braithwaite, 1978), was prevented. Since the Ca intake of both control and treated ewes was the same, this increased absorption was most probably due to an increased active transport of Ca.

All the available evidence suggests that normally, absorption of Ca is increased in the sheep only when skeletal Ca reserves have been considerably depleted and that in the pregnant and lactating ewe, this occurs in mid-lactation (Braithwaite *et al.* 1969, 1970; Braithwaite, 1974, 1975*b*, 1978). The fact that absorption of Ca can be increased in early lactation by treatment with 1α -OH- D_3 indicates that the normal regulatory mechanism controlling absorption was bypassed by the treatment. This is not unexpected, however, as 1α -OH- D_3 is rapidly converted to $1\alpha,25$ (OH) $_2D_3$ (Holick *et al.* 1976) by a process thought not to be subject to any regulatory control (Sansom *et al.* 1976).

It is unlikely that the increased urinary excretion of Ca occurred as a direct effect of the 1α -OH- D_3 treatment. In fact vitamin D-mediated Ca-binding proteins present in the kidney (DeLuca, 1975; Braithwaite & Glascock, 1976), might have been expected to reduce, not to increase this excretion (DeLuca, 1977). It is more likely that the increased excretion occurred merely as a result of the increased Ca concentration in the serum.

It is generally considered that $1\alpha,25$ (OH) $_2D_3$ increases both the rate of absorption of Ca from the intestine and the rate of mobilization of Ca from bone (Fraser & Kodicek, 1973; DeLuca, 1975, 1977). In the sheep there is normally an inverse relationship between these two processes (Braithwaite, 1975*b*; Braithwaite & Glascock, 1976). It is difficult, therefore, to understand how they could both increase at the same time unless there is a concomitant increase in the rate of Ca loss, or a continuously expanding pool of exchangeable Ca. In the present experiments, the rate of total loss of Ca increased only slightly and the size of the

exchangeable Ca pools remained constant. In consequence, the increased Ca absorption was accompanied by a decreased rate of bone mobilization, not an increased rate as is generally believed. This decrease in the bone mobilization rate probably reflects changes in the circulating levels of parathyroid hormone and calcitonin which occur as a result of increased serum Ca and P concentrations (Braithwaite, 1976). The situation, of course, may be different in the dairy cow at the onset of lactation when demands for Ca do increase suddenly and substantially. In these animals, both Ca absorption and bone mobilization may be increased. It must be pointed out, however, that the 1 α -OH-D₃ and 1 α ,25(OH)₂D₃ mediated increase in bone mobilization has been demonstrated in vivo only in animals maintained on Ca-deficient diets (Tanaka & DeLuca, 1971; Wong, Myrtle, Tsai & Norman, 1972; Holick *et al.* 1976). The present results suggest that such an increase is unlikely to occur when animals are maintained on adequate Ca intakes.

Rather surprisingly, the 1 α -OH-D₃ treatment resulted in a slight increase in the rate of bone accretion. Bone accretion normally remains constant in animals of a given age, and changes in skeletal retention are brought about by changes in the rate of bone resorption (Braithwaite & Riazuddin, 1971; Braithwaite, 1975*b*). However, the bone accretion rate has previously been shown to increase slightly in sheep during pregnancy and lactation and during treatment with growth hormone or oestrogen (Braithwaite *et al.* 1969, 1970, 1972; Braithwaite, 1975*a*) and there is also some evidence in man for its stimulation by 1 α -OH-D₃ (Pierides, Simpson, Ward, Ellis, Dewar & Kerr, 1976).

As the rate of secretion of P into the intestine was not measured, the true rate of P absorption could not be calculated. It is not possible therefore to conclude whether the increased rate of apparent P absorption (ingested P—faecal P) was effected by an increase in true absorption or to a decrease in intestinal secretion. However, the raised serum P concentration suggests that it is P absorption that is increased and this has recently been confirmed by P kinetic studies in sheep (Braithwaite, unpublished observations). In these studies, the rate of P absorption was markedly increased by treatment with 1 α -OH-D₃ and the rate of faecal endogenous secretion of P remained virtually unchanged.

It is concluded from these experiments that the major effect of 1 α -OH-D₃ is to stimulate the active absorption of Ca from the intestine. The beneficial effect of this compound in preventing post-parturient hypocalcaemia and reducing the incidence of parturient paresis in the dairy cow is therefore probably a result of this stimulation of active Ca transport.

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REFERENCES

- Agricultural Research Council (1965). *The Nutrient Requirements of Farm Livestock*, No. 2. Ruminants. London: HM Stationery Office.
- Aubert, J.-P. & Milhaud, G. (1960). *Biochim. biophys. Acta* **39**, 122.
- Braithwaite, G. D. (1974). *Br. J. Nutr.* **31**, 319.
- Braithwaite, G. D. (1975*a*). *Br. J. Nutr.* **33**, 309.
- Braithwaite, G. D. (1975*b*). *Br. J. Nutr.* **34**, 311.
- Braithwaite, G. D. (1976). *J. Dairy Res.* **43**, 501.
- Braithwaite, G. D. (1978). *Br. J. Nutr.* **39**, 213.
- Braithwaite, G. D. & Glascock, R. F. (1976). *Bienn. Rev. natn. Inst. Res. Dairy.*
- Braithwaite, G. D., Glascock, R. F. & Riazuddin, Sh. (1969). *Br. J. Nutr.* **23**, 827.
- Braithwaite, G. D., Glascock, R. F. & Riazuddin, Sh. (1970). *Br. J. Nutr.* **24**, 661.
- Braithwaite, G. D., Glascock, R. F. & Riazuddin, Sh. (1972). *Br. J. Nutr.* **28**, 269.
- Braithwaite, G. D. & Riazuddin, Sh. (1971). *Br. J. Nutr.* **26**, 215.
- Carlsson, A., Lindquist, M. & Magnusson, T. (1952). *Acta physiol. scand.* **26**, 212.

- Coie, C. R., Chamberlain, D. M., Hibbs, J. W., Pouden, W. D. & Smith, C. R. (1957). *J. Am. Vet. Med. Ass.* **130**, 298.
- DeLuca, H. F. (1975). *Acta orthop. scand.* **346**, 286.
- DeLuca, H. F. (1977). *Adv. Clin. Chem.* **19**, 125.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Fraser, D. R. & Kodicek, E. (1973). *Nature, New Biol.* **241**, 163.
- Hibbs, J. W. & Conrad, H. R. (1960). *J. Dairy Sci.* **43**, 1124.
- Hibbs, J. W. & Pouden, W. D. (1955). *J. Dairy Sci.* **38**, 65.
- Holick, M. F., Taleva, T. E., Holick, S. A., Schnoes, H. K., DeLuca, H. F. & Gallagher, B. M. (1976). *J. biol. Chem.* **251**, 1020.
- Manston, R. & Payne, J. M. (1964). *Br. vet. J.* **120**, 167.
- Olsen, W. G., Jorgensen, N. A., Schultz, L. H. & DeLuca, H. F. (1973). *J. Dairy Sci.* **56**, 889.
- Pierides, A. M., Simpson, W., Ward, M. K., Ellis, H. A., Dewar, J. H. & Kerr, D. N. S. (1976). *Lancet* **i**, 1092.
- Sansom, B. F., Allen, W. M., Davies, D. C., Hoare, M. N., Stenton, J. R. & Vagg, M. J. (1976). *Vet. Rec.* **99**, 310.
- Symonds, H. W., Manston, R., Payne, J. M. & Sansom, B. F. (1966). *Br. Vet. J.* **122**, 196.
- Tanaka, Y. & DeLuca, H. F. (1971). *Archs. Biochem. Biophys.* **146**, 574.
- Technicon Instruments Corporation (1967). *Technicon Method Sheet N-4B*. Tarry Town, New York: Technicon Instruments Corporation.
- Treacher, T. T. (1970). *J. Dairy Res.* **37**, 289.
- Wasserman, R. H. (1975). *Cornell Vet.* **65**, 3.
- Wong, R. G., Myrtle, H. C., Tsai, H. C. & Norman, A. W. (1972). *J. biol. Chem.* **247**, 5728.