The effect of hexoestrol on calcium metabolism in the sheep

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1. The effect of hexoestrol on calcium metabolism of wether lambs has been studied 5 weeks after implantation of the oestrogen by the use of ^{45}Ca combined with a balance technique.

2. Treatment resulted in significant increases in gain in body-weight, efficiency of food utilization and retention of Ca.

3. The increased retention of Ca resulted from an increase in the efficiency of absorption of dietary Ca and from a decrease in the rate of excretion of Ca into urine and faeces (faecal endogenous Ca).

4. The rate of accretion of Ca into bone was increased significantly by the treatment, but the rate of resorption of Ca from bone remained unchanged. The net result was an increase in skeletal retention of Ca.

5. The size of the slowly exchangeable pool of bone Ca also increased significantly.

6. The results suggest that hexoestrol promotes increased growth and Ca retention in ruminants by altering the metabolism of Ca to that of a younger more actively growing animal. This result would be consistent with an increased rate of secretion of growth hormone, the possibility of which is discussed together with the relationship between oestrogen and milk fever.

It has now been well established that suitable doses of oestrogen increase the rate of body-weight gain and efficiency of food utilization of sheep and cattle nearing maturity (Jordan, 1950; O'Mary, Pope, Wilson, Bray & Casida, 1951; Lamming, 1960; Jordan & Hanke, 1967), and that the increased body-weight gain is a result of an increase in the bone, moisture and muscle content of the carcass (Aitken & Crichton, 1956; Dodsworth, 1957). Oestrogen treatment has been reported to result also in an increased retention of calcium by ruminants (Whitehair, Gallup & Bell, 1953; Bell, Taylor, Murphree & Hobbs, 1955; Shroder & Hansard, 1958), but how this is brought about is not yet understood. The present work was undertaken to determine which of the various processes of Ca metabolism are modified by oestrogen treatment.

EXPERIMENTAL

Animals, housing and diet. Sixteen wethers, aged between 6 and 9 months, ten of which were Dorset-Horn and the other six Southdown, were used. Fifteen days before the start of the experiment the animals were placed in metabolism cages designed for the separate collection of urine and faeces. Animals of the same breed and similar weight were paired, one member of each pair being implanted with hexoestrol. The animals had free access to distilled water and were given a diet of hay and concentrates, the composition and Ca content of which are given in Table 1. The amount of food given to each animal was calculated according to its weight

Table 1. *Composition and calcium content of the diet given daily*

Ingredient	Amount (g/kg body-wt)	Ca content (mg/g)	Total Ca (mg/kg body-wt)
Hay	20	4·77	95·4
Barley	4	1·00	4·0
Flaked maize	2	0·05	0·1
Bran	1	0·72	0·7
Linseed-oil cake	0·5	2·90	1·4
Mineral mixture*	0·3	187·40	56·2
Vitamin mixture†	0·028	4·41	0·1
			157·9

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

at the time of hexoestrol implantation and, since the oestrogen-treated animals grew faster than the controls, the amount of Ca ingested daily/unit body-weight became progressively less than that of the controls (see Table 2).

Experimental procedure. One animal from each pair was implanted with one 15 mg pellet of hexoestrol (Hexoestrol Tablets for Implantation of Livestock; Boots Pure Drug Co., Nottingham) in the base of the ear by means of a Boots Cattle Implantation Instrument. This method of administration was chosen because it is used in farm practice, but we recognize that the daily dose of oestrogen was not known precisely. The daily rate of absorption from similar pellets implanted in a steer and a heifer has been found (Glascock & Hoekstra, 1958) to be about 30 μg per pellet and it seems reasonable to assume that it was similar in the present work.

Five weeks after implantation, the metabolism of Ca was studied by the method of Aubert & Milhaud (1960), which involves a combination of a balance and a radioactive technique. The application of this method to the sheep has been described in detail elsewhere (Braithwaite, Glascock & Riazuddin, 1969, 1970; Braithwaite & Riazuddin 1971).

RESULTS AND DISCUSSION

Mean values for the various processes of Ca metabolism are given in Table 2, together with their standard errors and results of tests of significance as determined by the *t* test for paired variants.

Body-weight gain and efficiency of food utilization. As expected, the treated wethers gained significantly more in body-weight than the untreated animals during the 5 weeks which followed implantation, thus showing a more efficient utilization of the food.

Retention of Ca. The treated animals retained considerably more Ca per unit body-weight than did the controls (Table 2), indicating that the effect of oestrogens on Ca metabolism is not merely a result of increased body size. Similar results have also been obtained by Whitehair *et al.* (1953), who used stilboestrol implants, and by Bell *et al.* (1955) and Shroder & Hansard (1958), who used orally administered stilboestrol.

Table 2. *A comparison of the metabolism of calcium in hexoestrol-treated and non-treated wethers* 6–9 *months of age and weighing* 30–40 *kg*

(Mean values for eight animals/group with their standard errors and results of tests of significance as determined by the *t* test for paired variants)

	Non-treated wethers		Hexoestrol-treated wethers		Significance of difference between means
	Mean	SE	Mean	SE	
Increase in live weight (in 5 weeks) as % of wt at time of implantation	13·4	5·5	21·6	5·7	***
Rapidly exchangeable pool of Ca (P) (mg/kg body-wt)	62·0	4·7	61·5	2·1	NS
Slowly exchangeable pool of Ca in bone (E) (mg/kg body-wt)	93·4	4·9	129·7	6·3	**
Rate of excretion of Ca in urine (mg/d kg body-wt)	7·1	1·6	2·5	0·4	*
Rate of excretion of Ca into intestine (faecal endogenous Ca) (mg/d kg body-wt)	23·2	1·8	20·1	1·6	*
Rate of accretion of Ca into bone (mg/d kg body-wt)	54·3	4·9	66·9	5·1	*
Rate of resorption of Ca from bone (mg/d kg body-wt)	32·2	3·5	30·3	2·4	NS
Rate of ingestion of Ca (mg/d kg body-wt)	152·6	11·8	147·6	12·4	*
Rate of loss of Ca in faeces† (mg/d kg body-wt)	123·5	6·6	108·5	8·4	**
Rate of absorption of Ca from intestine (mg/d kg body-wt)	52·3	5·9	59·2	5·1	NS
Ca absorption as % of Ca ingested	34·3	4·7	40·1	3·2	*
Ca balance (mg/d kg body-wt)	22·1	7·0	36·6	4·8	**

NS, not significant. * 0·05 > *P* > 0·01; ** 0·01 > *P* > 0·001; *** 0·001 > *P*.
† Sum of faecal endogenous Ca and unabsorbed Ca lost per d.

Absorption and excretion of Ca. Increased retention of Ca may be brought about either by increased intestinal absorption or decreased excretion. In young, growing sheep the amount of Ca absorbed/d is directly related to the amount ingested (Braithwaite & Riazuddin, 1971) which, as noted on page 270, varied with time after implantation. This is not, therefore, the quantity that should be used here for the purposes of comparison. If absorption is expressed as the proportion of dietary Ca ingested, small variations in daily intake have no effect and the difference between the treated and untreated animals then reflects the effect of hexoestrol on absorption of dietary Ca. The present results show that the proportion of dietary Ca absorbed by the experimental animals was significantly higher than that absorbed by the controls.

Excretion of Ca in both the urine and faeces (faecal endogenous Ca) was also significantly lower in the implanted sheep than in the controls. The increased retention of Ca after oestrogen treatment therefore must have resulted from a combination of increased absorption and decreased excretion. Shroder & Hansard (1958), however, found that, though absorption increased slightly in wethers treated with stilboestrol, this increase was not significant and the main effect of treatment was to markedly reduce the excretion of Ca into the intestine. In contrast with these observations, Braithwaite *et al.* (1969, 1970) found that faecal endogeneous excretion of Ca increased rather than decreased in ewes during pregnancy and lactation. However, in that work the effect of endogenous oestrogens on Ca excretion may have been masked by the effect of changes in levels of other hormones.

Skeletal metabolism. Any alteration in net retention of Ca by the body must occur almost wholly in the skeleton. Table 2 shows that the increased skeletal retention of Ca by hexoestrol-treated animals resulted from an increase in the rate per unit body-weight of Ca accretion into bone. The mean rate of resorption of Ca from bone was unchanged. Lotz & Comar (1958) concluded from their autoradiographic studies that the increased amount of bone of the femur of wethers treated daily with oestradiol-17β resulted from an inhibition of normal bone resorption, and Shroder & Hansard (1958) found that, though treatment with dietary stilboestrol increased the length of the femur, the difference between control and treated animals disappeared after correcting for body-weight. They therefore concluded that the increase resulted only from the greater rate of growth of the whole animal. This result, however, was not confirmed by their balance measurements for, even after correcting for body-weight, retention of Ca was very much greater (14·3 mg/d per kg) in the treated animals than in the controls (2·3 mg/d per kg).

Exchangeable Ca pools. Although the size of the rapidly exchangeable pool P was unaffected by hexoestrol, the size of the slowly exchangeable pool E of bone was significantly increased. This change, however, did not disturb the relationship between pool-size and the rate (V_{o^+}) of accretion of Ca into bone since the results from implanted animals were found still to satisfy the equation $E = 15{\cdot}3 + 1{\cdot}5\ V_{o^+}$ previously established for untreated animals of various ages (Braithwaite & Riazuddin, 1971).

Mode of action of hexoestrol in wethers. Several of the processes of Ca metabolism calculated to unit body-weight were increased by treatment with hexoestrol, which suggests that the oestrogen had an effect on Ca metabolism additional to that associated with an increase in body-weight.

The increases in size of the pool E, in the rate of accretion of Ca into bone and in the efficiency of absorption of dietary Ca all suggest that the effect of hexoestrol was to change the metabolism of Ca to that of a younger more actively growing animal (Braithwaite & Riazuddin, 1971). The mechanism by which the oestrogen brought about this change is at present largely a matter for speculation. Although a direct action on bone cannot be excluded, action by way of the thyroid or pituitary glands seems more probable. Both these glands selectively accumulate hexoestrol (Glascock & Smith, 1969) and both thyrocalcitonin and growth hormone cause an increase in skeletal retention of Ca. Since, however, thyrocalcitonin inhibits bone resorption (Copp, 1969) whereas growth hormone stimulates the accretion of Ca into bone (Ulrich, Reinhardt & Li, 1952), an increase in the rate of secretion of growth hormone rather than thyrocalcitonin would be more consistent with the findings in the present work (Table 2). Little appears to be known about the effect of oestrogens on the secretion of growth hormone in the male ruminant, but they are known to cause an increase in the plasma concentration of growth hormone in the ovariectomized rat (Dickerman & Meites, 1971).

Relationship between oestrogens and milk fever. It has recently been suggested (Stott, 1968) that milk fever in dairy cows is caused by the sudden increase in oestrogen levels at parturition resulting in a rapid increase in the rate of accretion of Ca into bone and a consequent depletion of the labile Ca pool E of bone.

Although milk fever is not often observed in sheep, results in this species may be relevant to the cow in which the disorder is common. Braithwaite *et al.* (1970) found that the rate of accretion of Ca into bone of the ewe was higher in lactation than in mid-pregnancy but, because of the considerable variation in results, they were unable to decide whether its rate increased in late pregnancy. In the present work there was an increase in the rate of accretion of Ca into bone after oestrogen treatment but this was only small and, furthermore, there was an increase, not a decrease, in the exchangeable Ca of bone. Thus it seems unlikely that alterations in the rate of bone accretion induced by oestrogen could alone be responsible for the onset of milk fever unless its rate increases to a much greater extent at parturition in cows prone to milk fever.

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