

## The effect of growth hormone on calcium metabolism in the sheep

By G. D. BRAITHWAITE

*National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

*(Received 17 December 1973 – Accepted 4 December 1974)*

1. The effect of subcutaneous administration of bovine growth hormone on calcium metabolism of nearly mature wether sheep has been studied by a combination of a radioactive technique and a nutrient balance technique.
2. Administration of growth hormone resulted in a significant increase in the rates of absorption of Ca, accretion of Ca into bone, resorption of Ca from bone and skeletal retention of Ca, and also in the sizes of the exchangeable Ca pools.
3. Retention of phosphorus was also significantly increased.
4. These changes suggest that the effect of growth hormone was to alter the Ca metabolism of nearly mature wethers to resemble that of younger, more actively growing animals.
5. Results are consistent with the theory that oestrogens may alter Ca metabolism of wethers by increasing growth hormone production.

Although it is well known that growth hormone is necessary for the normal growth and development of the skeleton, little work has been done to study its effect on the specific processes of calcium metabolism. Ulrich, Reinhardt & Li (1952) reported that the uptake of  $^{45}\text{Ca}$  by bone of young hypophysectomized rats was increased by growth hormone to the level found in intact controls. More recently, it has been reported that growth hormone administration increases the rates of Ca absorption, accretion of Ca into bone and Ca retention in adult man and dog (Henneman, Forbes, Moldawer, Dempsey & Carroll, 1960; Harris & Heaney, 1969; Heaney, Harris, Cockin & Weinberg, 1972).

On the basis of these reports, Braithwaite, Glascock & Riazuddin (1972) suggested that hexoestrol, a synthetic oestrogen, promotes increased Ca retention in wethers by stimulating the production of growth hormone.

The purpose of the present work was to study in detail the effect of growth hormone on Ca metabolism of nearly mature wether lambs.

### EXPERIMENTAL

*Animals, housing and diet.* Six 8-month-old Suffolk wethers weighing 35–40 kg were used for these studies. They were placed in metabolism cages, designed for the separate collection of urine and faeces, 1 month before the start of the experiment, to acclimatize to the experimental conditions. They were given a diet of hay and concentrates and had free access to distilled water. The amount of food given to each animal (Table 1) was calculated according to its weight at the start of the experiment. Since animals grew more rapidly after treatment with growth hormone, the amount of Ca and phosphorus ingested daily/unit body-weight decreased in the post-treatment period.

Table 1. *Composition, calcium and phosphorus content of the diet given daily to nearly mature wether lambs*

Ingredient	Amount given (g/kg body-wt)	Ca content (mg/g)	Total Ca (mg/kg body-wt)	P content (mg/g)	Total P (mg/kg body-wt)
Hay	20	4.70	94.0	1.60	32.0
Barley	4	0.79	3.2	4.17	16.7
Flaked maize	2	0.03	0.1	1.73	3.5
Bran	1	0.28	0.3	18.33	18.3
Linseed-oil cake	0.5	3.41	1.7	8.42	4.2
Mineral mixture*	0.3	170.68	51.2	124.17	37.3
Vitamin mixture†	0.07	12.83	0.9	2.16	0.2
Complete diet	—	—	151.4	—	112.2

\* Super Mindif (Boots Pure Drug Co., Nottingham).

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

*Experimental procedures.* All six animals were treated for 14 d with bovine growth hormone (NIH-GH-Bg; National Institute of Health, USA) at a dose rate of 0.5 mg/d per kg body-weight. The hormone, in saline, was administered by subcutaneous injection morning and evening in two equal parts. The effect of this dose on plasma growth hormone concentration was determined later in two of the sheep. Serial blood samples were taken by jugular cannula from 2 h before to 10 h after the injection of hormone, and plasma growth hormone concentrations were measured by radio-immunoassay (Hart, Flux, Andrews & McNeilly, 1974).

Ca kinetic studies were done by the method of Aubert & Milhaud (1960), modified for use with sheep (Braithwaite, Glascock & Riazuddin, 1969; Braithwaite & Riazuddin, 1971). These were done 1 month before treatment with growth hormone began, again 7 d after the start of the treatment and finally 1 month after the end of the treatment.

Since the purpose of these studies was to investigate a possible reversal, by growth hormone, of the changes in Ca metabolism that normally occur with increasing age (Braithwaite & Riazuddin, 1971), no control animals were included. It must be emphasized, however, that with this type of experimental design any period effects are overlooked.

Methods used for the determination of Ca and measurement of radioactivity in samples of blood, faeces and urine have been described previously (Braithwaite *et al.* 1969). Total phosphorus in ashed samples of food, faeces and urine was determined by the procedure of Fiske & Subbarow (1925) modified for use with an AutoAnalyzer (Technicon Instrument Corp., Basingstoke, Hants) (Technicon Instrument Corp., 1967).

#### RESULTS AND DISCUSSION

Fig. 1 shows that the concentration of growth hormone in the plasma increased markedly immediately after the subcutaneous injection of bovine growth hormone. The pre-injection concentration (1–5 ng/ml) was about normal for sheep (Trenkle, 1970a, 1971; Wallace & Bassett, 1970). The concentration after injection (60–110 ng/ml),

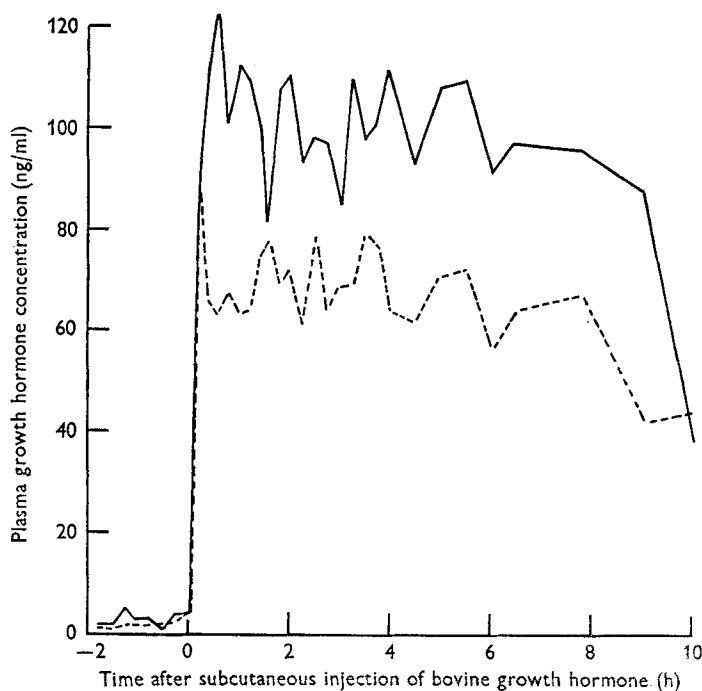


Fig. 1. Effect of subcutaneous injection of bovine growth hormone (0.25 mg/kg body-wt) on the individual plasma growth hormone concentrations for two nearly mature wether lambs.

although greater than normally found in ruminants, was not much greater than that for steers (up to 50 ng/ml) treated with oestrogen (Trenkle, 1970*b*) and was of a similar magnitude to that for sheep immediately after parturition (I. C. Hart, personal communication) and for the sheep foetus just before birth (Bassett, Thornburn & Wallace, 1970). The possibility exists, however, that such concentrations of growth hormone may disturb the secretion and action of other hormones (Wallace & Bassett, 1966) and it must also be emphasized that the response of sheep to bovine growth hormone may not be the same as that to ovine growth hormone although there is a reasonable degree of structural similarity between the two hormones.

Since the half-time of intravenously injected growth hormone is of the order of 7–15 min (Wallace & Bassett, 1970; Trenkle, 1971) uptake into the plasma from the subcutaneous injection must have been only gradual to account for the prolonged (8–9 h) high plasma concentration. As the growth hormone was injected daily in two equal parts, early morning and late evening, a fairly constant high plasma concentration must have been maintained throughout the whole experimental period.

The effects of growth hormone on the various processes of Ca metabolism of nearly mature wethers are shown in Table 2.

*Retention, absorption and excretion.* Retention of both Ca and P was significantly increased by growth hormone treatment. The increase in Ca retention was the result of an increase in the efficiency of absorption of dietary Ca; endogenous excretion of Ca in the urine and into the intestine (faecal endogenous excretion) was unchanged. The

Table 2. *Calcium metabolism in 8-month-old wether lambs, before, during and after treatment with bovine growth hormone (0.5 mg/d per kg body-wt)*

(Mean values for six sheep)

	1 month before start of treatment	7 d after start of treatment	1 month after end of treatment	Standard error (residual mean square)	Difference between means required for statistical significance at $P < 0.001$
Weight (kg)	36.3	36.8	43.8	0.3	1.9
Rapidly exchangeable pool of Ca (mg/kg body-wt)	56.2	70.0	49.0	1.6	10.2
Slowly exchangeable pool of Ca in bone (mg/kg body-wt)	80.9	145.8	103.3	6.4	41.9
Rate of excretion of Ca in urine (mg/d per kg body-wt)	8.7	7.0	6.7	0.4	2.8
Rate of excretion of Ca into intestine (faecal endogenous Ca) (mg/d per kg body-wt)	21.1	21.9	18.8	0.6	3.6
Rate of accretion of Ca into bone (mg/d per kg body-wt)	44.6	80.0	64.0	4.0	26.1
Rate of resorption of Ca from bone (mg/d per kg body-wt)	45.9	62.8	61.8	2.8	19.7
Rate of ingestion of Ca (mg/d per kg body-wt)	136.9	130.4	111.1	1.5	9.8
Rate of loss of Ca in faeces* (mg/d per kg body-wt)	129.7	106.2	102.1	0.8	14.6
Rate of absorption of Ca from intestine (mg/d per kg body-wt)	28.3	46.1	27.8	1.5	9.6
Ca absorption: Ca ingested	0.207	0.353	0.250	0.11	0.71
Ca balance (mg/d per kg body-wt)	-1.5	+17.2	+2.3	1.7	11.1
Rate of ingestion of phosphorus (mg/d per kg body-wt)	103.4	101.6	84.8	0.8	5.5
Rate of loss of P in faeces (mg/d per kg body-wt)	94.2	77.1	72.4	2.3	14.9
Rate of excretion of P in urine (mg/d per kg body-wt)	2.1	2.1	1.4	0.6	3.9
P balance (mg/d per kg body-wt)	+7.1	+22.4	+11.0	1.5	12.9

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

increase in retention of P was probably also the result of an increased efficiency of absorption but results do not allow a definite conclusion to be reached as faecal endogenous excretion of P was not measured.

One month after the end of the hormone treatment the rates of absorption of Ca and retention of Ca and P had returned almost to the values found before treatment.

*Skeletal metabolism.* The results in Table 2 indicate that skeletal turnover of Ca increased markedly as a result of growth hormone treatment. The rate of accretion of Ca into bone increased more than the rate of resorption of Ca from bone; skeletal retention of Ca was therefore increased. This effect of growth hormone on Ca metabolism is interesting as increased skeletal retention is usually brought about in sheep by a decrease in the rate of resorption of Ca from bone and the rate of accretion of Ca into bone tends to remain constant (Braithwaite & Riazuddin, 1971; Braithwaite, 1974). Growth hormone does, however, cause similar increases in accretion of Ca

into bone in man and dog (Henneman *et al.* 1960; Harris & Heaney, 1969; Heaney *et al.* 1972).

Although the rate of Ca accretion into bone had decreased considerably 1 month after the end of the hormone treatment, it still remained higher than in the pretreatment period. The reason for this persistent action of growth hormone on the skeleton is uncertain but may be due to osteoblasts and osteoclasts produced during treatment, continuing to function for some considerable time after withdrawal of the treatment.

*Exchangeable Ca pools.* Both the rapidly exchangeable pool of Ca and the slowly exchangeable pool of Ca in bone were significantly increased by treatment with growth hormone. Although the rapidly exchangeable pool had decreased to below its pretreatment level 1 month after the end of the treatment, the slowly exchangeable bone pool remained enlarged. The slowly exchangeable pool of Ca in bone ( $E$ ) has been found to be directly related to the rate of accretion of Ca into bone ( $V_0^+$ ) in sheep of different ages and breeds (Braithwaite & Riazuddin, 1971). This relationship was found also to hold for growth-hormone-treated sheep ( $P < 0.001$ ), the regression equation  $E = 14.1 + 1.5 V_0^+$ , was similar to that ( $E = 15.3 + 1.5 V_0^+$ ) previously reported (Braithwaite & Riazuddin, 1971).

The enlarged size of  $E$ , 1 month after the withdrawal of the treatment is probably only a consequence of the high  $V_0^+$  at this time.

*Mechanism of action of growth hormone.* Increases in the rates of Ca retention, Ca absorption and bone turnover and in the sizes of exchangeable pools of Ca during treatment suggest that administration of growth hormone reverses the effects of age on Ca metabolism (Braithwaite & Riazuddin, 1971) and results in younger, more actively growing animals. In fact, Ca metabolism of the sheep before treatment resembled that of animals of a similar age (9 months) in the experiments of Braithwaite & Riazuddin (1971) but during treatment, resembled that of animals only 3–6 months old.

These results suggest that for sheep the changes in Ca metabolism which are found during the first year of life may be due to a gradual decrease in production of growth hormone or a decrease in the sensitivity to growth hormone of target tissues. Reports on the relationship between growth hormone status and age in ruminants are conflicting. Armstrong & Hansel (1956) and Curl, Fennell, Zinn & Albin (1968) reported a gradual decrease with age in the anterior pituitary growth hormone content/unit body-weight in growing cattle and Eaton, Klosterman & Johnson (1968) found that the concentrations of circulating growth hormone in weanling calves were twice those of the mothers. Bassett *et al.* (1970), Dev & Lasley (1969) and Purchas, Macmillan & Hafs (1970), however, were unable to find any significant relationship between growth hormone status and age in their experiments.

The mechanism by which growth hormone alters Ca metabolism is uncertain. Finkelstein & Schachter (1962) reported that it was necessary for the vitamin D-mediated active transport of Ca across the rat intestine. Since metabolites of vitamin D have recently been implicated in skeletal metabolism (Trummel, Raisz, Blunt & DeLuca, 1969; Wong, Myrtle, Tsai & Norman, 1972; Reynolds, Holick & DeLuca, 1973), growth hormone may also have an important function in the skeleton. On the

other hand, evidence has recently accumulated which suggests that growth hormone does not itself stimulate linear growth but rather induces the formation of secondary growth-promoting factors, the somatomedins (Van Wyk, Underwood, Lister & Marshall, 1973). It is possible therefore that the action of growth hormone on Ca metabolism might be mediated by somatomedins.

*Relationship between the effects of growth hormone and oestrogen on Ca metabolism.* Braithwaite *et al.* (1972) found that hexoestrol administration altered the Ca metabolism of nearly mature wether sheep to resemble that of younger animals and they suggested that the oestrogen might exert its effect by way of the thyroid or pituitary glands. Trenkle (1970*b*) reported that stilboestrol treatment markedly increased the plasma growth hormone concentration of finishing steers. In the present experiments, growth hormone administration had a similar effect on the Ca metabolism of nearly mature wether sheep to that reported for hexoestrol. Results therefore support the suggestion that oestrogen might alter Ca metabolism by increasing the secretion of growth hormone.

The author thanks Dr R. F. Glascock for his interest and advice, Dr I. C. Hart for the growth hormone determinations, Miss S. Neville, Mr C. T. Ray and Mr B. Woods for technical assistance and Mr R. Ellis for care of the experimental animals. The gift of bovine growth hormone from the Endocrinology Study Section, National Institute of Health, USA, is gratefully acknowledged.

## REFERENCES

- Armstrong, D. T. & Hansel, W. (1956). *J. Anim. Sci.* **15**, 640.  
 Aubert, J.-P. & Milhaud, G. (1960). *Biochim. biophys. Acta* **39**, 122.  
 Bassett, J. M., Thornburn, G. D. & Wallace, A. L. C. (1970). *J. Endocr.* **48**, 251.  
 Braithwaite, G. D. (1974). *Br. J. Nutr.* **31**, 319.  
 Braithwaite, G. D., Glascock, R. F. & Riazuddin, Sh. (1969). *Br. J. Nutr.* **23**, 827.  
 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.  
 Curl, S. E., Fennell, M. A., Zinn, D. W. & Albin, R. C. (1968). *J. Anim. Sci.* **27**, 1011.  
 Dev, V. G. & Lasley, J. F. (1969). *J. Anim. Sci.* **29**, 384.  
 Eaton, L. W., Klosterman, E. W. & Johnson, R. R. (1968). *J. Anim. Sci.* **27**, 1785.  
 Finkelstein, J. D. & Schachter, D. (1962). *Am. J. Physiol.* **203**, 873.  
 Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.  
 Harris, W. H. & Heaney, R. P. (1969). *Nature, Lond.* **223**, 403.  
 Hart, I. C., Flux, D. S., Andrews, P. & McNeilly, A. S. (1974). *Horm. Metab. Res.* (In the Press.)  
 Heaney, R. P., Harris, W. H., Cockin, J. & Weinberg, E. H. (1972). *Calc. Tiss. Res.* **10**, 14.  
 Henneman, P. H., Forbes, A. P., Moldawer, M., Dempsey, E. F. & Carroll, E. L. (1960). *J. clin. Invest.* **39**, 1223.  
 Purchas, R. W., Macmillan, K. L. & Hafs, H. D. (1970). *J. Anim. Sci.* **31**, 358.  
 Reynolds, J. J., Holick, M. F. & DeLuca, H. F. (1973). *Calc. Tiss. Res.* **12**, 295.  
 Technicon Instruments Corp. (1967). *Technicon Method Sheet N-4B*. Tarrytown, New York: Technicon Instruments Corp.  
 Trenkle, A. (1970*a*). *Proc. Soc. exp. Biol. Med.* **133**, 1018.  
 Trenkle, A. (1970*b*). *J. Anim. Sci.* **31**, 389.  
 Trenkle, A. (1971). *Proc. Soc. exp. Biol. Med.* **136**, 51.  
 Trummel, C. L., Raisz, L. G., Blunt, J. W. & DeLuca, H. F. (1969). *Science, N.Y.* **163**, 1450.  
 Ulrich, F., Reinhardt, W. O. & Li, C. H. (1952). *Acta endocr., Copenh.* **10**, 117.  
 Van Wyk, J. J., Underwood, L. E., Lister, R. C. & Marshall, R. N. (1973). *Am. J. Dis. Child.* **126**, 705.  
 Wallace, A. L. C. & Bassett, J. M. (1966). *Metabolism* **15**, 95.  
 Wallace, A. L. C. & Bassett, J. M. (1970). *J. Endocr.* **47**, 21.  
 Wong, R. G., Myrtle, J. F., Tsai, H. C. & Norman, A. W. (1972). *J. biol. Chem.* **247**, 5728.